

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C12N 15/62, C07K 13/00, G01N 33/50, C12N 1/19	A1	(11) International Publication Number: WO 94/25607 (43) International Publication Date: 10 November 1994 (10.11.94)
(21) International Application Number: PCT/US94/04379 (22) International Filing Date: 20 April 1994 (20.04.94) (30) Priority Data: 08/052,207 23 April 1993 (23.04.93) US (71) Applicant: UNIVERSITY OF IOWA RESEARCH FOUNDATION [US/US]; Technology Innovation Center, Oakdale, IA 52242 (US). (72) Inventor: TEEM, John, L.; 1244 E. College, Iowa City, IA 52245 (US). (74) Agents: SEASE NEBEL, Heidi et al.; Zarley, McKee, Thomte, Voorhees & Sease, Suite 3200, 801 Grand Avenue, Des Moines, IA 50309 (US).		(81) Designated States: AU, CA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD OF ASSAYING FOR DRUGS WHICH RESTORE FUNCTION OF MUTANT CFTR PROTEIN		
(57) Abstract A method of identifying potentially useful treatments which restore the function of CFTR or other related protein mutations is disclosed. The method involves creation of yeast STE6-CFTR chimeric sequences which upon introduction of a desired mutation inhibit expression of the yeast STE6 gene. A yeast assay (STE6Δ) transformed to include the hybrid chimeras is then exposed to a potential treatment for the mutation. A treatment which restores the function of the STE6 gene and thereby allows mating of the yeast strain is indicative of a potential remedial treatment for the mutation in this selected protein.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

**Title: METHOD OF ASSAYING FOR DRUGS WHICH RESTORE
 FUNCTION OF MUTANT CFTR PROTEIN**

BACKGROUND OF THE INVENTION

Cystic fibrosis is a human genetic disease of the secretory epithelia. Although the survival rate of those suffering with cystic fibrosis has improved in recent years, the median age for patient survival is still only about twenty five to thirty years despite intensive supportive and prophylactic treatment. Today cystic fibrosis remains the most common congenital disease among Caucasians, where it has a prevalence of about one in two thousand live births, and is uniformly fatal. Nearly all patients suffering from the disease develop chronic progressive disease of the respiratory system, the most common cause of death being pulmonary disease. Also, in the majority of cases, pancreatic dysfunction occurs; hepatobiliary and genitourinary diseases are also frequent. Because of the multi-system clinical manifestations of the disease, current methods of treatment for the disease have focused on therapeutic approaches to reduce the symptoms of cystic fibrosis.

United States Patent No. 5,100,647 to Agus, et al, discloses a method for treating cystic fibrosis by administration of the compound sparteine (dodecahydro-7, 14methano-2H, 6H-di-pyrido [1,2-a: 1',2'-e] [1,5] diazocine), acting as a direct exogenous activator of chloride conductants in epithelial airways. United States Patent No. 5,179,001 to Young, et al, discloses a method of treating pulmonary complications associated with cystic fibrosis caused by the gram negative bacterium Pseudomonas aeruginosa. United States

- 2 -

Patent No. 4,826,679 to Roy relates to an oral composition for alleviating digestive manifestation in persons afflicted with cystic fibrosis comprising a therapeutic amount of taurine. Despite much advancement in the treatment of the symptoms of cystic fibrosis very little has been done to effectively "cure" the disease at a genetic level.

One method of gene therapy proposed is United States Patent No. 5,149,797 disclosing a method of site specific alteration of RNA and production of encoded polypeptides. This invention is drawn to correcting the abnormal mRNA present in individual cells, cleaving the mRNA by site directed RNAase followed by introduction of the appropriate oligoribonucleotide followed by endogenous RNA ligase and thus production of a wild-type mRNA encoding a normal protein product which then may be translated to produce the correct protein.

Cystic fibrosis is characterized at the genetic level by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The CFTR protein is a member of a superfamily of structurally related membrane proteins named the traffic ATPase or ATP-binding cassette (ABC) transporters. Other members of this superfamily include the multi-drug resistance (MDR) or P-glycoprotein, bovine adenylyl cyclase, the yeast STE6 protein, as well as several bacterial amino acid transport proteins (Riordan, et al, 1989; Hyde, et al, 1990). The principle distinguishing feature of this superfamily of proteins is a highly conserved nucleotide-binding domain (NBD).

CFTR is a protein of approximately 1480 amino acids consisting of two repeated motifs, each

- 3 -

comprising 6 transmembrane segments and a nucleotide-binding domain. The two motifs are separated by a large polar R-domain containing multiple potential phosphorylation sites.

Many members of the ABC transport superfamily transport small molecules across biological membranes in an ATP dependent manner. However, CFTR is a Cl^- channel regulated by phosphorylation and by cytosolic ATP (Anderson, et al, "Nucleoside Triphosphates are Required to Open CFTR Chloride Channel" *Cell*, 67, 775-784). The two nucleotide-binding domains present in CFTR play a central role in the function of CFTR Cl^- channels; they interact directly with ATP to open the CFTR Cl^- channel and ATP hydrolysis may be required for this effect. The importance of the NBD's is further emphasized by the large number of cystic fibrosis associated mutations that have been found in the domains. The most common cystic fibrosis associated mutation, accounting for approximately 68% of the cystic fibrosis chromosomes is deletion of a phenylalanine at position 508 (ΔF508) in the middle of NBD1.

This mutation is characterized by a loss of apical membrane Cl^- channel activity in the epithelial cells leading to chronic progressive disease of the respiratory system. The ΔF508 mutation causes loss of apical membrane Cl^- channel activity affecting the CFTR protein in two ways. First, the mutation causes defective processing and hence mislocalization of the mutant protein. This is presumed to occur from misfolding of the mutant protein so that it fails to exit from the endoplasmic reticulum and progress to the Golgi complex in the apical membrane. The resulting Cl^-

- 4 -

transport defect associated with the $\Delta F508$ is thus largely a consequence of the absence of functional CFTR at the plasma membrane. Second the $\Delta F508$ mutation affects the function of the Cl^- channel. Single channel analysis of CFTR $\Delta F508$ suggests that although the mutant protein is functional, it has an open state probability (P_o) that is one third that of wild-type CFTR.

Defective trafficking of CFTR $\Delta F508$ can be partially reversed if cells are grown at a lower temperature, indicating that mutant protein is still functional. When CFTR $\Delta F508$ is produced at a reduced temperature (less than $37^\circ C$) some of the mutant protein transits the Golgi complex normally and is localized correctly in the plasma membrane. (See Denning, G.M.; Anderson, M.P.; Amara, J.; Marshall, J.; Smith, A.E.; and Welsh, M.J.); "Processing of Mutant CFTR ($\Delta F508$) is Temperature Sensitive." *Nature*, 358, 761-764. Since CFTR $\Delta F508$ retains partial function, a possible therapy for cystic fibrosis may involve pharmacologic intervention designed to correct the processing defects so that more CFTR $\Delta F508$ reaches the plasma membrane. Potential pharmacologic intervention would represent a non gene-therapy approach for the treatment of cystic fibrosis.

As can be seen, a great need exists in the art for methods of treating cystic fibrosis that do not concentrate solely on the symptoms produced by the disease, but rather treat the disease at a cellular level by attempting to restore function to the mutant product. In light of the scientific potential for pharmacologic treatments, a method for identification of new candidate drugs for cystic

- 5 -

fibrosis treatment is warranted. This is complicated, however, by the fact that a screening method to identify treatments that reverse the CFTR $\Delta F508$ Cl^- transport defect in mammalian cells is impractical; large numbers of chemical compounds cannot easily be screened for an activity that restores the cAMP-stimulated Cl^- transport of mutant CFTR.

It is an object of this invention to provide an in vitro method of screening pharmaceutical compositions, and further mutations for their ability to restore function to defective ABC transport proteins resulting from mutations such as CFTR $\Delta F508$, by simple means of a yeast mating assay.

It is another object of the invention to construct chimeric gene sequences which encode the yeast STE6 gene and the CFTR gene for screening of treatments which will restore function to the mutant proteins.

It is another object of the invention to construct chimeric gene sequences which encode the yeast STE6 gene and the P-glycoprotein gene (or other ABC transporter genes) for screening treatments which will inhibit the function of the chimeric protein.

Yet another object of the invention is to provide a method for identifying potentially useful compounds for further useful study in the treatment of disease by means of a yeast mating assay.

Other objects of the invention will become obvious from the description of the invention which follows.

- 6 -

SUMMARY OF THE INVENTION

This invention relates to a method for screening treatments which restore the function of mutant CFTR by simple means of a yeast cell mating assay. According to the present invention, chimeric genetic sequences which encode the yeast a-factor transporter STE6 and CFTR or another member of the related superfamily such as P-glycoprotein are constructed whereby a segment of the CFTR genetic sequence is exchanged for a corresponding segment of the STE6 genetic sequence to produce a product which will complement a yeast *ste6* Δ mutation but is non-functional upon introduction of a desired mutation into the CFTR sequence.

The resulting chimeras are then transformed into a yeast strain which has the STE6 gene deleted (*ste6* Δ). The yeast strain is then exposed to the desired drug or treatment to be screened and the strain is assayed for the presence of restoration of mating, which indicates a functional chimera. The invention discloses several chimeric sequences useful for screening for utility of treatments and reversing the effects of the CFTR Δ F508 mutation which is associated with cystic fibrosis.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 represents the amino acid sequence alignment of NBD1 of STE6 and CFTR.

Figure 2 depicts the STE6-CFTR chimeric constructs and their mating efficiency.

Figure 3 is a graphical depiction of a yeast assay according to the present invention which presents a potential cystic fibrosis treatment.

- 7 -

Figure 4a-g is the gene sequence for chimera H4.

Figure 5a-g is the gene sequence for chimera H5.

Figure 6a-g is the gene sequence for chimera H6.

DETAILED DESCRIPTION OF THE INVENTION

The STE6 gene of Saccharomyces cerevisiae encodes an integral membrane protein that functions to transport the peptide a-factor out of the cell during mating. (Kuchler and Thorner, 1992), "Functional expression of human MDR1 in yeast Saccharomyces cerevisiae." Proc. Natl. Acad. Sci., USA 89, 2303-2306. Deletion of the STE6 gene from yeast (ste6 Δ) results in a sterile phenotype. According to the present invention, chimeric STE6 genes were constructed in which segments of the NBD1 of STE6 were substituted by the analogous NBD1 sequences from CFTR.

Selection for sites of exchange was accomplished by comparing the known amino acid sequences of CFTR and STE6. The CFTR and STE6 amino acid sequences were aligned by use of the "BEST FIT" algorithm for the comparison of DNA and amino acid sequences obtained from the University of Wisconsin and found in the GCG Sequence Analysis Package.

Figure 1 represents an amino acid sequence alignment of the NBD for proteins CFTR and STE6. The CFTR amino acid sequence is depicted on top illustrating amino acids 442 through 578; the corresponding analogous STE6 amino acid sequence is depicted below illustrating amino acids 376 through 536. Corresponding amino acids are indicated by

- 8 -

solid vertical bars and give an indication of potential junctions for the genetic constructs.

Table 1 indicates the portion of CFTR amino acids sequence inserted into STE6 for the different hybrids.

TABLE 1

<u>HYBRID IDENTIFIER</u>	<u>PORTION OF CFTR AMINO ACID SEQUENCE</u>
H1	D443-Y577
H2	D443-F508
H3	F494-F508
H4	F494-I546
H5	F494-L558
H6	F494-Y577

The H1 STE6-CFTR chimeric gene was constructed with a DNA sequence coding for the entire NBD1 of STE6 (from N377 to A535) replaced by the corresponding region of CFTR (D443 to Y557). (See Figure 1.) H2 replaced the amino terminal half of NBD and H6 replaced the carboxy terminal half. The remaining constructs were variations between these two extremes. (See Figure 2.) The construction of plasmids with these sequences used methods which are well known to those of skill in the art however the following description is included merely for purposes of illustration and is not intended to limit in any way the invention.

PLASMID CONSTRUCTIONS

Plasmid RFG416 (a gift from Rick Gaber, Northwestern University) is a single copy CEN plasmid with the selectable marker URA3 and the pUC19 polylinker region. A 6.5 kb Sall-SacI fragment containing the STE6 gene was subcloned from STE6-2 μ (a gift from John McGrath, Massachusetts

- 9 -

Institute of Technology) into the Sall and SacI sites of the vector RFG416 to produce plasmid JTS6. The yeast TRP1 gene (on a 0.85 kb BglII-EcoRI DNA fragment) was inserted at nucleotide position 1356 of STE6 NBD1, resulting in JTS6T.

To construct the H1 STE6-CFTR hybrid plasmid, a 441 bp DNA fragment containing the CFTR NBD1 region was synthesized using two STE6-CFTR oligonucleotide primers (primer 1, 5'CCTTCGGAAGCAGTCCTGAAAGATAT-3'; primer 2, 5'-GATGAACAATATCTAGGTATCCAAAA-3') and CFTR cDNA template DNA in a polymerase chain reaction (PCR) (Ho, et al., 1989). PCR reactions were performed with a Temp-Tronic thermal cycler (Barnstead/Thermolyne). Oligonucleotide primers 1 and 2 encoded fusion junctions of STE6 L375 to CFTR K442 and CFTR 1578 to STE6 D537, respectively, found in the H1 STE6-CFTR hybrid gene. The CFTR portion of each primer is underlined. STE6 DNA flanking NBD1 was added to each end of the 441 bp fragment by PCR, resulting in a 867 bp DNA fragment consisting of 419 bp of CFTR DNA (encoding CFTR amino acids K442-L578) that is flanked at the 5' end by 168 bp of STE6 DNA (encoding STE6 amino acids K319-L375) and 280 bp of STE6 DNA at the 3' end (encoding STE6 amino acids D537-G640). Plasmid construction of H1 was performed by cotransformation (Ito, et al, 1983) of yeast strain JPY201 with the 867 bp DNA fragment and 3 µg of plasmid JTS6T and selection of transformants on SD-URA (yeast nitrogen base supplemented with all amino acids except uracil). Homologous recombination between the STE6 DNA sequences at each end of the 867 bp DNA fragment with the STE6 gene on the plasmid results in the targeted integration (Orr-Weaver, et al, 1981) of the CFTR sequences into

- 10 -

NBD1 of STE6 on plasmid JTS6T and the consequent loss of the TRP1 gene. Recombinants containing the desired STE6-CFTR hybrid gene were identified as trp auxotrophs at a frequency of about 1% among the transformants. Plasmid DNA was prepared from trp transformants and the structure of the STE6-CFTR gene was confirmed by DNA sequencing analysis. STE6-CFTR hybrid genes H2-H6 were similarly constructed using the following oligonucleotide primers for constructing the appropriate STE6CFTR junctions; STE6 Q440/CFTR F494, 5'-CACCGTCGTAGAACAGTTTTCTGGATTA-3'; CFTR G509/STE6 S457, 5'-CCGAATCTGTTGAACCAAAGATGATATTT-3'; CFTR G550/STE6 G509, 5'-TTGTTGTTGCCCGCCACTCAGTGTGATTC-3'; CFTR R560/STE6 A519, 5' ATCTCTGATGAATGCTCTTGCTAAA-GAAAT-3'.

The gene sequences for H4, H5, and H6 are included in Figures 4-6.

The resulting plasmids were then transformed into a yeast strain with the STE6 gene deleted (ste6 Δ) to test for complementation by means of a yeast mating assay.

YEAST CELL MATING ASSAY

Several types of variations of assays may be used and are commonly known to those of skill in the art. The following description is not intended to limit in any way the invention.

The yeast strains JPY201 (MATa ste6 Δ :HIS3, gal2, ura3-52, lys2-801, trp1, leu2-3,112, his3 Δ 200) and 22-2D (MATa, ura352, leu2-3,112, trp1) were used for all mating experiments. JPY201 contains a STE6 deletion (including NBD1 and extending beyond the termination codon) and replacement with the

- 11 -

yeast HIS3 gene (McGrath and Varshavsky, 1989). Quantitative mating assays were performed as in Trueheart, et al (1987).

Transformants of JPY201 containing each STE6-CFTR chimera were grown to log phase in 0.1% glucose SD-URA media. From each culture, 3×10^6 cells were mixed with an equal number of 22-2D cells grown in YPD media and collected by filtration onto a Millipore filter, which was then placed upon a YPD plate for 4 hours at 30°C. Cells were resuspended, sonicated briefly, and plated from serial dilutions onto SD+LEU/TRP (yeast nitrogen base supplemented with leucine and tryptophan). Diploid colonies were counted after 3 days at 30°C. Control strains STE6 wild-type and *ste6* Δ consisted of JPY201 transformed with plasmids JTS6 and JTS6T, respectively. For qualitative petri dish mating assays, JPY201 transformants containing STE6-CFTR chimeras were grown as patches on SD-URA media and then replica printed to YPD media on a lawn of 22-2D cells. Following incubation at 30°C for 8 hr, the plate containing the mating cells was replica printed to SD-LEU/TRP and incubated 3 days at 30°C to allow growth of diploid colonies.

The complementation results are given in Figure 2. As can be seen yeast transformants containing the H1 sequence were unable to complement the *ste6* Δ mutation, indicating a nonfunctional STE6 chimera. Similarly, the H2 sequence replacing the amino half only was nonfunctional. See Figures 1 and 2. These results suggest that the amino terminal region of NBD1 from CFTR cannot substitute for that of STE6.

In contrast, the STE6-CFTR chimeras containing the central part of NBD1 from CFTR (H3 and H4, See

- 12 -

Figures 1 and 2) maintained a-factor transport activity comparable with the wild-type STE6 yeast strain. This suggests that amino acid residues between the conserved Walker A and B motifs (Walker, et al, 1982) although different in the NBD1 of CFTR and STE6 provide a similar overall structure and function. When the chimeras included progressively larger segments of the carboxy-terminal region of NBD1 from CFTR (H5 and H6) mating efficiency was reduced. Yeast transformed with the hybrid gene H5 which contained the substitution of 74 residues of STE6 NBD1 (from R441 to I516) by that from CFTR (from F494 to L558) mated at 12% efficiency of wild-type STE6. Mating efficiency was further reduced in H6 to background levels. However, a low level of mating was observed when the H6 gene construct was expressed on a high copy number plasmid.

INTRODUCTION OF THE Δ F508 MUTATION

A previous study has suggested that missense mutations in STE6 that were analogous to CF-associated mutations in NBD1 of CFTR result in defective a-factor transport, however, single amino acid deletions analogous to Δ F508 within the central region of STE6 NBD1 had no effect on the STE6 function. (Berkower and Michaelis, "Mutational Analysis of the Yeast a-factor Transporter STE6; a Member of the ATP Binding Cassette (ABC Protein Superfamily)," *EMBO J* 10, 3777-3785 (1991)).

Contrary to this finding, it was found that by incorporating CFTR into NBD1 of the STE6 gene and also including the Δ F508 mutation within the CFTR segment, function of the STE6 chimeric gene was inhibited. The chimeric sequence constructs H3, H4,

- 13 -

H5, and H6 which demonstrated complementation of the *ste6* Δ yeast strain were also used to assess the effect of the Δ F508 mutation. The Δ F508 mutation was introduced into H3, H4, H5, and H6 (resulting in H3- Δ F508, H4- Δ F508, H5- Δ F508, and H6- Δ F508 respectively). Figure 2 shows the effect of the Δ F508 mutation on a-factor transport as assessed by the quantitative yeast cell mating assay.

As seen from the results in Figure 2, yeast transformed with the H3- Δ F508 chimera mated with an efficiency equal to the H3 control (no F508 mutation). A decrease in mating efficiency (40%) was observed with the strain containing the H4- Δ F508 chimera relative to the H4 control. (Sequence I.D. No. 1). The Δ F508 mutation lowered mating efficiency by 80 fold in the H5- Δ F508 chimera as compared with H5 (Sequence I.D. No. 3). The H6- Δ F508 chimera displayed a comparable decrease in mating as compared to H6 (Sequence I.D. No. 5) efficiency due to the Δ F508 mutation when expressed on a high copy number plasmid, (and relative to the H6 chimera gene also on high copy number plasmid vector). For use in accordance with the present invention, expression of the H6- Δ F508 gene on a high copy number plasmid would be required.

Based on the foregoing junction analysis, the portion of CFTR most promising for substitution into STE6 include the amino acid identity locations between a portion of NBD1 from amino acid 444 up to amino acid 509 through 577. As earlier seen, the segment from 443 to 557 was inactive as was the portion from 443 to 508. Preferred ranges include from amino acid 494 to 577 including: sequence I.D. No. 1, CFTR amino acid F494-I546; Sequence I.D. No.

- 14 -

3, F494-L558; Sequence I.D. No. 5 CFTR amino acid F494-Y577. Several areas of identity between the two aligned sequences as demonstrated in Figure 1 exist within this range of CFTR amino acid substitutions and will represent other chimeric sequences useful for the present invention.

YEAST MATING ASSAY TO IDENTIFY
COMPOUNDS THAT CORRECT THE Δ F508 DEFECT

In accordance with the present invention, a yeast strain transformed to contain the discussed chimeric sequences will be used in a yeast mating assay to identify compounds which will correct the affect of the mutation as indicated by an increase in mating. Such an assay may be widely varied by those of skill in the art. Generally, the transformed strain will be combined with a strain of the alpha mating type on a suspension of yeast cells spread on the surface of agar medium in the petri dish. These two strains would be unable to mate due to the Δ F508 mutation.

A compound is then introduced to the medium. For example a plant screening method may be performed by placing a small disc of leaf material removed by means of a paper punch from a plant and placed on the surface of the agar media. Plant compounds will diffuse from the leaf and interact with the yeast strains.

Another method would be to use a filter paper which has been soaked in the desired chemical and then placed on the yeast suspension. Further, other desired mutations can be introduced into the CFTR region to attempt to identify a revertant mutation and a corresponding protein for further study. It is to be understood that any form of exposing the

- 15 -

yeast strain to the desired treatment may be used including modifying external conditions such as temperature.

The mating plate is then incubated for 24 hours to allow yeast cell mating to occur. If a compound corrects the molecular defect associated with H5- Δ F508 transporter then a-factor is transported allowing mating between the two strains, resulting in diploid cells. The mating plate is then replica printed to a selective media plate to allow growth of diploid colonies.

For example strain A could be - MATa, STE6::HIS3, ura3-52, lys2, trp1-298, his3-200, leu2-3,112 <plasmid H5- Δ F508, URA3, CEN>; strain B - MAT α , ura3-52, trp1, leu2-3,112.

The selective media used would then be lacking lysine and uracil. Consequently strains A and B could not grow on this media (strain A has the lys2 mutation and strain B has the ura3-52 mutation; these mutations prevent them from going on media without lysine and uracil respectively), however diploids produced as a result of the fusion between strain A and B can grow (diploids contain the genomes of both haploids and consequently has the complementing URA3 and LYS2 wild-type allele).

Production of yeast diploid cells in the area surrounding the treatment disc would be a strong indicator of presence of compound(s) which correct the Δ F508 defect. A number of yeast mating type assays may be developed easily by those of skill in the art and the foregoing description is not intended to limit in any way the invention.

- 16 -

EXAMPLE 1REVERTANTS OF Δ F508 MATING DEFECT IN YEAST
(MUTATION IN R553-L558 REGION TO SUPPRESS Δ F508 IN NBD1)

The R553-L558 amino acid region of CFTR is of great interest in CFTR and other trafficking ATPase's as it is directly adjacent to the conserved LSGGQ linker sequence of NBD1, postulated to function as a transducer of signals between the hydrophobic domains and the NBD's (Mimura, et al, 1991). It was hypothesized that a possible treatment to correct the F508 mutation could be interaction of the region of the NBD polypeptide containing F508, with the R553-L558 region. The only difference between the H5 chimera and the H4 chimera is 6 amino acids in the R553-L558 region. This region is also not present in the H3 chimera in which the Δ F508 mutation had no effect. Possibly there is an interaction between the two regions of the NBD required for correct folding or function (or both) of the NBD. Thus the method of the present invention was used to test whether a mutation in the region R553-L558 might suppress the adverse effects of the Δ F508 in NBD1.

To isolate revertants of the H5- Δ F508 mating defect, the H5- Δ F508 construct was mutagenized in the R553-L558 region in vitro by site-specific oligonucleotide mutagenesis (Ho, et al, "Site-Directed Mutagenesis by Overlap Extension Using the Polymerase Chain Reaction Gene" 77,51-59 (1989)). The mutagenesis method was designed to introduce random amino acid substitutions at single codon positions within the R553-L558 region of the H5- Δ F508 plasmid. The procedure allowed 20 possible substitutions at each amino acid position within

- 17 -

R553-L558. The mutagenized DNA was transformed into yeast, and transformant colonies were analyzed by cell mating assays on petri dishes to identify colonies with a mating efficiency higher than the unmutagenized H5- Δ F508 control. Two colonies identified displayed higher efficiencies of mating within the first 20 mutant transformants analyzed. These yeast transformants each contained an H5- Δ F508 plasmid with a mutation at amino acid R553 of CFTR; in one case, R553 was replaced by methionine (H5- Δ F508/R553M), and in the other plasmid, R553 was replaced by glutamine (H5- Δ F508/R553Q). It is possible that other mutations within the R553-L558 region of the H5-F508 plasmid could also result in increased mating efficiency; however, the R553Q and R553M mutants were analyzed in greater detail without further mutagenesis of the R553-L558 region.

The relative mating efficiency of the Δ F508 revertant strains are shown in Table 2, with results expressed relative to the original H5 strain. Whereas the mating efficiency of the H5- Δ F508 yeast strain is approximately 1% of the H5 strain, yeast containing the H5- Δ F508/R553Q and H5- Δ F508/R553M plasmids mated at 3% and 32%, respectively. The revertant mutations, therefore, partially suppress the Δ F508 mating defect. The R553M mutation alone had little effect on H5 (H5-R553M); when this mutant was transformed into yeast, no further increase in mating efficiency was observed as compared with yeast containing H5 (Table 2). In accordance with this invention, mutations were found which suppress the effects of the Δ F508 mutation.

- 18 -

TABLE 2Relative Mating Efficiency of Δ F508 Revertants

<u>Genotype</u>	<u>Mating Efficiency Relative to H5 (%)</u>
H5-R553M	77.4 = 3.7
H5- Δ F508/R553M	34.2 = 7.8
H5- Δ F508/R553Q	3.2 = 0.8
H5- Δ F508	1.1 = 0.5

Mating efficiencies were determined by quantitative mating assays and are expressed as a percentage relative to H5 (100%). Data are means = SEM.

EXAMPLE 2TESTING OF THE R553M and R553Q
MUTATIONS IN THE MAMMALIAN SYSTEM

To determine whether the mutations which corrected the Δ F508 mutation would also correct the defect in human CFTR Δ F508, the R553Q and R553M mutations were introduced into CFTR Δ F508 cDNA and mammalian cells were transfected with these constructs to determine whether the revertant mutations would correct the defect in cAMP-regulated Cl^- transport of CFTR Δ F508.

The revertants R553Q and R553M were tested for their effects on mislocalization and the channel gating of CFTR Δ F508. The identified revertants modified both of these effects. Mutation of R553 to methionine partially corrected the processing of CFTR Δ F508 as assessed by three criteria; it produced fully glycosylated protein as indicated by electrophoretic separation on SDS polyacrylamide gel; it increased the appearance of the mutant protein in the plasma membrane as determined immunocytochemically; and it produced functional

- 19 -

channels in the plasma membrane when measured by the SPQ halide efflux assay. These results demonstrate the functional utility of the yeast model system for identifying revertants of CF-associated mutations of the NBD1 of CFTR and its use in drug screening for pharmacologic compounds that correct the mating defect. The mutations discovered could be used to identify peptides which may be of utility in reversing the effects of the $\Delta F508$ mutation.

EXAMPLE 3

R553Q AND R553M SUPPRESS THE CFTR $\Delta F508$ ANION TRANSPORT DEFECT

The effect of the R553Q and R553M mutations on CFTR function was determined by a cAMP-stimulated halide efflux using the halide-sensitive fluorophore 6-methoxy-N-(3-sulfopropyl)-quinolinium (SPQ) (Illsley and Verkman, 1987). Expression of CFTR cDNA containing either the R553Q or the R553M mutation alone (without the $\Delta F508$ mutation) in HeLa cells generated cAMP-stimulated halide efflux like wild-type CFTR. Cells expressing the $\Delta F508$ allele in this recombinant system showed little, if any, cAMP-stimulated halide efflux. However, when the mutations R553Q and R553M were introduced into CFTR $\Delta F508$ (CFTR $\Delta F508$ /R553Q and CFTR $\Delta F508$ /R553M, respectively), cAMP-dependent anion permeability was restored. These results indicate that the CFTR Cl⁻ channel defect observed with the $\Delta F508$ mutant could be suppressed by either R553 mutation.

Cl⁻ transport by CFTR $\Delta F508$ containing the R553Q and R553M mutations would be detected only if the processing defect of CFTR $\Delta F508$ was suppressed. To determine further whether the suppressor

- 20 -

mutations correct the processing defect associated with the $\Delta F508$ mutation, the glycosylation patterns of CFTR and the various mutants expressed in HeLa cells were examined. CFTR is a glycoprotein that undergoes progressive glycosylation, resulting in three bands that migrate at different rates on an SDS-polyacrylamide gel. Band A is the most rapidly migrating and represents the nascent, unglycosylated protein; band B has an intermediate rate of migration and a pattern of core glycosylation consistent with processing in the endoplasmic reticulum; band C migrates most slowly and has a pattern of mature glycosylation consistent with processing in the Golgi complex. CFTR $\Delta F508$ was only presented as the unglycosylated band A and the core glycosylated band B protein, consistent with its failure to traverse the Golgi complex and reach the plasma membrane.

We first examined the glycosylation state of CFTR in HeLa cells expressing CFTR cDNA containing either the R553Q and R553M mutations alone (without the $\Delta F508$ mutation). Band C was present in cells expressing wild-type CFTR and also mutant CFTR containing either the R553Q or R553M mutation. In contrast, only bands A and B were present in cells expressing CFTR $\Delta F508$. Thus, the R553Q and R553M mutations alone do not affect the glycosylation of CFTR.

In cells transfected with the CFTR $\Delta F508/R553M$, a small increase in band C was detectable as compared with CFTR $\Delta F508$. This result was observed in three separate experiments. Band C was not able to be consistently detected in cells expressing CFTR $\Delta F508/R553Q$ possibly owing to

- 21 -

limitations in the sensitivity of the assay. However, a detectable increase in Band C with CFTR $\Delta F508/R553M$ as compared with CFTR $\Delta F508$ occurred.

To assess further the ability of suppressor mutations to correct the mislocalization defect of CFTR $\Delta F508$, immunocytochemistry was used to detect CFTR at the cell surface in HeLa cells expressing wild-type or mutant CFTR. An antibody specific to an external epitope in the first extracellular loop of CFTR was used. (Denning, et al, 1992b). Wild-type CFTR is generally detected at the surface of unpermeabilized HeLa cells. In contrast, no CFTR was detectable at the surface of cells expressing CFTR $\Delta F508$. However, when cells expressed CFTR $\Delta F508/R553M$, CFTR was detected at the plasma membrane. For CFTR $\Delta F508/R553Q$, plasma membrane staining of CFTR was weak and variable and could not be demonstrated consistently. Only nonspecific staining was observed with preimmune serum or in the absence of CFTR. These results were consistent with the observation that more CFTR $\Delta F508/R553M$ than CFTR $\Delta F508/R553Q$ was found in the band C form and suggest that the amount of CFTR $\Delta F508/R553Q$ at the plasma membrane is exceedingly low.

SINGLE-CHANNEL ANALYSIS OF CFTR $\Delta F508/R553Q$ CFTR

To determine whether the suppressor mutations altered the single-channel properties of CFTR $\Delta F508$, the revertant mutant CFTR $\Delta F508/R553Q$ was expressed in HeLa cells and analyzed with the patch-clamp technique. Single-channel analysis of CFTR $\Delta F508$ indicated that the P_o of the CFTR $\Delta F508$ Cl^- channel was reduced as compared with wild-type CFTR. Denning, et al, (1992a) reported that CFTR $\Delta F508$ had

- 22 -

a P_o of 0.13 ± 0.01 (after incubation at reduced temperature), compared with a value of 0.34 ± 0.02 for wild-type, and Dalemans, et al, (1991) reported that the P_o of CFTR $\Delta F508$ Cl^- channels ranged from 0.05 to 0.10, compared with a range of 0.22 to 0.35 for wild type.

As expected from the response observed in the SPQ studies, CFTR/R553Q and CFTR $\Delta F508$ /R553Q formed functional Cl^- channels. Inside-out membrane patches from either mutant showed no Cl^- channel activity under basal conditions. However, addition of protein kinase A(PKA) and ATP to the cytosolic surface of the membrane patch activated channels in both cases; both agents were required to activate the channels ($n = 6$ for each mutant). Single-channel events were recorded from +60 mV to -120 mV in increments of 20 mV. The channels were Cl^- selective as indicated by the reversal potentials ($E = 20$ mV: $E_{Cl^-} = 27$ mV). CFTR/R553Q had a single-channel slope conductance (from -120 mV to 0 mV) of 9.8 ± 0.5 pS ($n = 7$), and CFTR $\Delta F508$ /R553Q had a conductance of 10.2 ± 0.2 pS ($n = 11$). The current measured at -100 mV for CFTR/R553Q (-1.1 ± 0.1 pA, $n = 6$) and CFTR $\Delta F508$ /R553Q (-1.2 ± 0.0 pA, $n = 5$) was not different from the current measured for wild-type CFTR (-1.1 ± 0.0 pA, $n = 7$). Thus, these data indicate that neither mutation altered the single-channel conductive properties of the CFTR Cl^- channel, nor did the mutations abolish the regulation by PKA. The results also indicate that, as predicted by the functional analysis of CFTR $\Delta F508$ /R553Q by the SPQ halide efflux assay above, CFTR $\Delta F508$ /R553Q is localized in the plasma membrane.

- 23 -

Following activation with PKA and ATP, we measured the P_o for CFTR/R553Q and CFTR $\Delta F508$ /R553Q at different MgATP concentrations. As the concentration of MgATP increased, both CFTR/R553Q and CFTR $\Delta F508$ /R553Q spent more time in the open state. The P_o of CFTR/R553Q Cl^- channels was similar to that previously reported for wild-type CFTR Cl^- channels (Anderson and Welsh, 1992). However, at 1mM ATP, the P_o of CFTR $\Delta F508$ /R553Q (0.29 ± 0.02 , $n = 7$) was greater than that of CFTR $\Delta F508$ Cl^- channels (0.13 ± 0.01 , $n = 4$). Thus, the R553Q mutation corrected the functional defect in gating of the CFTR $\Delta F508$ Cl^- channels.

YEAST MATING ASSAY WITH PLANT EXTRACT SCREENING

A suspension of yeast cells (strains A and B) was spread upon the surface of agar media in a petri dish.

The suspension consisted of equal proportions of two haploid yeast strains. Strain A - MAT α , STE6::HIS3, ura3-52, lys2, trp1-298, his3-200, leu2-3,112 <plasmid H5- $\Delta F508$, URA3, CEN>; strain B - MAT α , ura3-52, trp1, leu2-3,112.

Yeast strains A and B are of the a and α mating types respectively, and can mate to form yeast diploids. Strain A contains the STE6/CFTR hybrid a-factor transporter gene H5- $\Delta F508$ (Teem, et al, 1993). The hybrid a-factor transporter encoded by the H5- $\Delta F508$ gene is defective as a result of the $\Delta F508$ mutation. Strain A is therefore unable to transport the mating pheromone a-factor efficiently. Since the transport of the a-factor pheromone is required for yeast cell mating, mating between strains A and B is inefficient.

- 24 -

The agar media is one rich in nutrients (nonselective for the growth of strains A and B) necessary for the optimal mating of yeast.

An extract prepared from the plant genus *Grindelia* was prepared by grinding fresh *Grindelia* leaves in a mixture of ethanol and water (1:1). This extract contains the fraction of plant material soluble in a solution of 1:1 ethanol and water. Small filter paper discs were prepared using a standard paper punch and Whatman filter paper #2. A filter paper disc containing the *Grindelia* extract was prepared by adding 10 μ l. of extract to the disc, and then allowing the disc to air dry. Additional extract was added in 10 μ l aliquots, allowing the disc to dry after each application until a total of 30 μ l. was added to the disc. The disc was then placed upon the surface of the media, on top of the yeast cells.

The mating plate was incubated for 24 hours at 30°C to allow yeast cell mating to occur.

Chemical compounds that diffuse out of the filter paper disc into the agar will come in contact with the mating cells. A component of the *Grindelia* extract promotes mating of strains A and B.

The mating plate was replica printed to a selective media plate that is incubated at 30°C for three days to allow the growth of diploid colonies.

The selective media is lacking lysine and uracil. Consequently, strains A and B cannot grow on this media (strain A has the *lys2* mutation and strain B has the *ura3-52* mutation: these mutations prevent them from growing on media without lysine and uracil respectively). However, diploids produced as a result of the fusion between strain A

- 25 -

and B can grow (diploids contain the genomes of both haploids and thus contain the complementing URA3 and LYS2 wild-type alleles). Production of yeast diploid cells in the area surrounding a leaf disc would be a strong indication that the leaf disc contained a compound which corrects the $\Delta F508$ defect. Diploids cells were easily detected by replica printing the yeast cells from the mating plate, onto another petri dish plate containing media which is selective for the growth of the diploid cells. This media will allow the diploid yeast cells to grow and form colonies, while preventing the growth of the haploid (unmated) strain A and B yeast cells. The frequency of diploid colonies produced on the selective plate is an indirect measurement of the ability of strain A to transport a-factor and mate.

A high density of yeast colonies on the selective media plate occurred in the location corresponding to the position where the filter paper disc containing the Grindelia extract occurred on the mating plate. This is clearly distinguishable from the low level of diploid colonies which are produced by the mating of the two yeast strains in the absence of a chemical compound that corrects the $\Delta F508$ defect. (See Figure 3.)

As can be seen in Figure 3 the extract reverses the effect of the $\Delta F508$ mutation indicating its potential for further study.

Thus it can be seen the invention accomplishes all of its objectives.

- 26 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: University of Iowa Research Foundation.
- (ii) TITLE OF INVENTION: Method of Assaying for Drugs which Restore Function of CFTR Mutations
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Zarley, McKee, Thomte, Voorhees & Sease
 - (B) STREET: 801 Grand Ave. Suite 3200
 - (C) CITY: Des Moines
 - (D) STATE: Iowa
 - (E) COUNTRY: US
 - (F) ZIP: 50309
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vii) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/US94/
 - (B) FILING DATE: 20-APR-1994
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/052,207
 - (B) FILING DATE: 04-APR-1993
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Nebel, Heidi S.
 - (B) REGISTRATION NUMBER: 37,719
 - (C) REFERENCE/DOCKET NUMBER: Uirf 93-50
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 515-288-3667
 - (B) TELEFAX: 515-288-1338

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3840 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 27 -

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..3840

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1317..1488
 (D) OTHER INFORMATION: /note= "Substituted analogous sequence from human CFTR gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG	48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg	
1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA ACA GGC CTA GTG CCG GCA ATT ACT TCT ATC CTG ACG	144
Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr	
35 40 45	
GGC AGA GTG TTC GAT CTA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT	192
Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His	
50 55 60	
CAA GGT TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA	240
Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala	
65 70 75 80	
CTT GGT GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG	288
Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp	
85 90 95	
ATG CAC ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG	336
Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu	
100 105 110	

- 28 -

GAG Glu	GCA Ala	TAT Tyr 115	TTG Leu	GAG Glu	GAA Glu	AAG Lys	CCA Pro 120	ATG Met	GAA Glu	TGG Trp	TAC Tyr 125	GAC Asp	AAT Asn	AAT Asn	GAA Glu	384
AAA Lys	TTG Leu	TTA Leu	GGA Gly	GAT Asp	TTT Phe	ACT Thr 135	CAA Gln	ATC Ile	AAC Asn	AGA Arg	TGT Cys 140	GTG Val	GAA Glu	GAG Glu	CTA Leu	432
AGA Arg 145	TCA Ser	AGC Ser	TCC Ser	GCA Ala	GAG Ala 150	GCA Ala	TCA Ser	GCC Ala	ATA Ile	ACT Thr 155	TTC Phe	CAG Gln	AAT Asn	TTA Leu	GTT Val 160	480
GCA Ala	ATA Ile	TGT Cys	GCG Ala 165	CTT Leu	CTG Leu	GGG Gly	ACG Thr	TCA Ser	TTC Phe 170	TAC Tyr	TAT Tyr	TCT Ser	TGG Trp	TCA Ser 175	TTA Leu	528
ACT Thr	TTA Leu	ATT Ile 180	ATT Ile	CTT Leu	TGC Cys	AGC Ser	TCT Ser 185	CCA Pro	ATA Ile	ATC Ile	ACA Thr	TTT Phe 190	TTT Phe	GCA Ala	GTG Val	576
GTG Val	TTT Phe	TCC Ser 195	AGA Arg	ATG Met	ATT Ile	CAT His 200	GTA Val	TAT Tyr	TCA Ser	GAG Glu	AAG Lys 205	GAG Glu	AAT Asn	TCT Ser	GAA Glu	624
ACG Thr 210	AGT Ser	AAA Lys	GCA Ala	GCC Ala	CAA Gln 215	TTA Leu	CTT Leu	ACA Thr	TGG Trp	TCG Ser	ATG Met 220	AAT Asn	GCC Ala	GCT Ala	CAA Gln	672
TTA Leu 225	GTG Val	AGA Arg	TTA Leu	TAT Tyr	TGT Cys 230	ACA Thr	CAA Gln	CGT Arg	CTA Leu	GAA Glu 235	AGG Arg	AAA Lys	AAA Lys	TTC Phe	AAG Lys 240	720
GAA Glu	ATC Ile	ATA Ile	CTA Leu 245	AAT Asn	TGT Cys	AAC Asn	ACT Thr	TTC Phe 250	TTC Phe	ATC Ile	AAG Lys	AGT Ser	TGC Cys	TTT Phe 255	TTT Phe	768
GTT Val	GCT Ala	GCA Ala	AAC Asn 260	GCT Ala	GGG Gly	ATC Ile	TTG Leu	AGA Arg 265	TTT Phe	TTG Leu	ACG Thr	TTG Leu	ACT Thr	ATG Met	TTT Phe	816
GTA Val	CAG Gln	GGA Gly 275	TTC Phe	TGG Trp	TTT Phe	GGT Gly	TCC Ser 280	GCA Ala	ATG Met	ATC Ile	AAA Lys	AAG Lys	GGC Gly	AAG Lys	CTG Leu	864
AAC Asn	ATT Ile	AAC Asn	GAT Asp	GTA Val	ATC Ile	ACT Thr 295	TGC Cys	TTC Phe	CAT His	TCA Ser	TGC Cys 300	ATT Ile	ATG Met	CTG Leu	GGC Gly	912
TCG Ser 305	ACT Thr	TTA Leu	AAT Asn	AAT Asn	ACA Thr 310	TTA Leu	CAC His	CAA Gln	ATA Ile	GTT Val 315	GTT Val	CTT Leu	CAA Gln	AAA Lys	GGC Gly 320	960

- 29 -

GGA GTG GCT ATG GAA AAA ATC ATG ACT CTA TTA AAA GAT GGA TCC AAG	1008
Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys	
325 330 335	
CGA AAT CCT TTA AAT AAA ACT GTA GCC CAC CAA TTT CCA CTA GAT TAT	1056
Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr	
340 345 350	
GCC ACC AGT GAT CTA ACA TTT GCT AAT GTT TCG TTT TCT TAT CCA AGC	1104
Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser	
355 360 365	
AGA CCT TCG GAA GCA GTT TTA AAG AAC GTT AGT TTA AAT TTC TCT GCA	1152
Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala	
370 375 380	
GGA CAA TTT ACT TTC ATA GTA GGA AAA TCA GGC TCA GGT AAA TCT ACA	1200
Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr	
385 390 395 400	
TTA TCC AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA	1248
Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile	
405 410 415	
TCT ATC AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT	1296
Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile	
420 425 430	
GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC	1344
Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr	
435 440 445	
ATT AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC	1392
Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr	
450 455 460	
AGA AGC GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT	1440
Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe	
465 470 475 480	
GCA GAG AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT	1488
Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser	
485 490 495	
GGC GGG CAA CAA CAA AGA GTT GCT ATA GCA CGT GCA TTC ATC AGA GAT	1536
Gly Gly Gln Gln Gln Arg Val Ala Ile Ala Arg Ala Phe Ile Arg Asp	
500 505 510	
ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT	1584
Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val	
515 520 525	

- 30 -

CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys 530 535 540	1632
ACT ACA ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp 545 550 555 560	1680
TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln 565 570 575	1728
TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC Ser Glu Leu Leu Ala Asp Pro Thr Thr Phe Ser Thr Trp Tyr His 580 585 590	1776
CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr 595 600 605	1824
GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu 610 615 620	1872
ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr 625 630 635 640	1920
GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val 645 650 655	1968
AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu 660 665 670	2016
AAA CAA CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser 675 680 685	2064
ATT ATT CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys 690 695 700	2112
ATT CTA ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn 705 710 715 720	2160
CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro 725 730 735	2208

- 31 -

TCC ACG GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser 740 745 750	2256
CTT CTT GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala 755 760 765	2304
AAA GGA TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu 770 775 780	2352
AGA AAT GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe 785 790 795 800	2400
TCT GGT GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn 805 810 815	2448
GAT TTG CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met 820 825 830	2496
ACT AGT TTC GTT ACC GTA TCA ACG ATT GGA CTA ATT TGG GCG TTA GTA Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val 835 840 845	2544
TCG GGC TGG AAG TTA AGT TTG GTT TGT ATT TCG ATG TTT CCA CTC ATA Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile 850 855 860	2592
ATT ATA TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr 865 870 875 880	2640
GAT TAT AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile 885 890 895	2688
GTC ACT AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe 900 905 910	2736
CAA TTG ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser 915 920 925	2784
AAA CGC GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile 930 935 940	2832

- 32 -

GTC ATG TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val 945 950 955 960	2880
ATG ATT CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu 965 970 975	2928
TTA TTA TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro 980 985 990	2976
GAT ATA AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu 995 1000 1005	3024
GAT GAA AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr 1010 1015 1020	3072
GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1025 1030 1035 1040	3120
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1045 1050 1055	3168
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1060 1065 1070	3216
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu 1075 1080 1085	3264
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly 1090 1095 1100	3312
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1105 1110 1115 1120	3360
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1125 1130 1135	3408
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1140 1145 1150	3456

- 33 -

GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648
ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val 1220 1225 1230	3696
ATA ACG CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val 1235 1240 1245	3744
CTT AAA GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr 1250 1255 1260	3792
AAT AAT CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser 1265 1270 1275	3840

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1279 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg
1				5					10					15	
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile
			20					25					30		
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr
		35					40					45			

- 34 -

Gly 50	Arg	Val	Phe	Asp	Leu	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His
Gln 65	Gly	Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala 80
Leu	Gly	Ala	Ala	Ser 85	Val	Pro	Val	Met	Trp 90	Leu	Ser	Leu	Thr	Ser 95	Trp
Met	His	Ile	Gly 100	Glu	Arg	Gln	Gly	Phe 105	Arg	Ile	Arg	Ser	Gln 110	Ile	Leu
Glu	Ala	Tyr 115	Leu	Glu	Glu	Lys	Pro 120	Met	Glu	Trp	Tyr	Asp 125	Asn	Asn	Glu
Lys	Leu 130	Leu	Gly	Asp	Phe	Thr 135	Gln	Ile	Asn	Arg	Cys 140	Val	Glu	Glu	Leu
Arg 145	Ser	Ser	Ser	Ala	Glu 150	Ala	Ser	Ala	Ile	Thr 155	Phe	Gln	Asn	Leu	Val 160
Ala	Ile	Cys	Ala	Leu 165	Leu	Gly	Thr	Ser	Phe 170	Tyr	Tyr	Ser	Trp	Ser 175	Leu
Thr	Leu	Ile	Ile 180	Leu	Cys	Ser	Ser	Pro 185	Ile	Ile	Thr	Phe	Phe 190	Ala	Val
Val	Phe	Ser 195	Arg	Met	Ile	His	Val 200	Tyr	Ser	Glu	Lys 205	Glu	Asn	Ser	Glu
Thr	Ser 210	Lys	Ala	Ala	Gln	Leu 215	Leu	Thr	Trp	Ser	Met 220	Asn	Ala	Ala	Gln
Leu 225	Val	Arg	Leu	Tyr	Cys 230	Thr	Gln	Arg	Leu	Glu 235	Arg	Lys	Lys	Phe	Lys 240
Glu	Ile	Ile	Leu	Asn 245	Cys	Asn	Thr	Phe	Phe 250	Ile	Lys	Ser	Cys	Phe 255	Phe
Val	Ala	Ala	Asn 260	Ala	Gly	Ile	Leu	Arg 265	Phe	Leu	Thr	Leu	Thr 270	Met	Phe
Val	Gln	Gly 275	Phe	Trp	Phe	Gly	Ser 280	Ala	Met	Ile	Lys	Lys 285	Gly	Lys	Leu
Asn	Ile 290	Asn	Asp	Val	Ile	Thr 295	Cys	Phe	His	Ser	Cys 300	Ile	Met	Leu	Gly
Ser 305	Thr	Leu	Asn	Asn	Thr 310	Leu	His	Gln	Ile	Val 315	Val	Leu	Gln	Lys	Gly 320

- 35 -

Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys
 325 330 335
 Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr
 340 345 350
 Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser
 355 360 365
 Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala
 370 375 380
 Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr
 385 390 395 400
 Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile
 405 410 415
 Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile
 420 425 430
 Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr
 435 440 445
 Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr
 450 455 460
 Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe
 465 470 475 480
 Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser
 485 490 495
 Gly Gly Gln Gln Gln Arg Val Ala Ile Ala Arg Ala Phe Ile Arg Asp
 500 505 510
 Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val
 515 520 525
 His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys
 530 535 540
 Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp
 545 550 555 560
 Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln
 565 570 575
 Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His
 580 585 590

- 36 -

Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr
 595 600 605
 Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu
 610 615 620
 Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr
 625 630 635 640
 Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val
 645 650 655
 Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu
 660 665 670
 Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser
 675 680 685
 Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys
 690 695 700
 Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn
 705 710 715 720
 Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro
 725 730 735
 Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser
 740 745 750
 Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala
 755 760 765
 Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu
 770 775 780
 Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe
 785 790 795 800
 Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn
 805 810 815
 Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met
 820 825 830
 Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val
 835 840 845
 Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile
 850 855 860

- 37 -

Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr
 865 870 875 880
 Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile
 885 890 895
 Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe
 900 905 910
 Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser
 915 920 925
 Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile
 930 935 940
 Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val
 945 950 955 960
 Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu
 965 970 975
 Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro
 980 985 990
 Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu
 995 1000 1005
 Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr
 1010 1015 1020
 Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile
 1025 1030 1035 1040
 Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala
 1045 1050 1055
 Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu
 1060 1065 1070
 Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu
 1075 1080 1085
 Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly
 1090 1095 1100
 Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser
 1105 1110 1115 1120
 Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn
 1125 1130 1135

- 38 -

```

Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp
      1140                      1145                      1150
Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln
      1155                      1160                      1165
Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala
      1170                      1175                      1180
Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu
      1185                      1190                      1195                      1200
Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile
      1205                      1210                      1215
Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val
      1220                      1225                      1230
Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val
      1235                      1240                      1245
Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr
      1250                      1255                      1260
Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser
      1265                      1270                      1275

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3840 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..3840
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1318..1521

- 39 -

(D) OTHER INFORMATION: /note= "Substituted analogous
sequence from human CFTR gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG	48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg	
1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA ACA GGC CTA GTG CCG GCA ATT ACT TCT ATC CTG ACG	144
Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr	
35 40 45	
GGC AGA GTG TTC GAT CTA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT	192
Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His	
50 55 60	
CAA GGT TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA	240
Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala	
65 70 75 80	
CTT GGT GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG	288
Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp	
85 90 95	
ATG CAC ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG	336
Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu	
100 105 110	
GAG GCA TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA	384
Glu Ala Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu	
115 120 125	
AAA TTG TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA	432
Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu	
130 135 140	
AGA TCA AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT	480
Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val	
145 150 155 160	
GCA ATA TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA	528
Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu	
165 170 175	

- 40 -

ACT	TTA	ATT	ATT	CTT	TGC	AGC	TCT	CCA	ATA	ATC	ACA	TTT	TTT	GCA	GTG	576
Thr	Leu	Ile	Ile	Leu	Cys	Ser	Ser	Pro	Ile	Ile	Thr	Phe	Phe	Ala	Val	
			180					185						190		
GTG	TTT	TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	624
Val	Phe	Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	
		195					200					205				
ACG	AGT	AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	672
Thr	Ser	Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	
	210				215						220					
TTA	GTG	AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	720
Leu	Val	Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	
225					230					235					240	
GAA	ATC	ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	768
Glu	Ile	Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	
				245					250					255		
GTT	GCT	GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	816
Val	Ala	Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	
			260					265					270			
GTA	CAG	GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	864
Val	Gln	Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	
		275					280					285				
AAC	ATT	AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	912
Asn	Ile	Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	
	290					295					300					
TCG	ACT	TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	960
Ser	Thr	Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	
					310					315					320	
GGA	GTG	GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	1008
Gly	Val	Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	
				325					330				335			
CGA	AAT	CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	1056
Arg	Asn	Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	
			340					345					350			
GCC	ACC	AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	1104
Ala	Thr	Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	
		355					360					365				
AGA	CCT	TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	1152
Arg	Pro	Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	
	370					375					380					

- 41 -

GGA CAA TTT ACT TTC ATA GTA GGA AAA TCA GGC TCA GGT AAA TCT ACA Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr 385 390 395 400	1200
TTA TCC AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA Leu Ser Asn Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile 405 410 415	1248
TCT ATC AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile 420 425 430	1296
GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr 435 440 445	1344
ATT AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr 450 455 460	1392
AGA AGC GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe 465 470 475 480	1440
GCA GAG AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser 485 490 495	1488
GGA GGT CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA TTC ATC AGA GAT Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp 500 505 510	1536
ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val 515 520 525	1584
CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys 530 535 540	1632
ACT ACA ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp 545 550 555 560	1680
TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln 565 570 575	1728
TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His 580 585 590	1776

- 42 -

CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr 595 600 605	1824
GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu 610 615 620	1872
ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr 625 630 635 640	1920
GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val 645 650 655	1968
AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu 660 665 670	2016
AAA CAA CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC Lys Gln Gln Lys Asn Thr Glu Ser Thr Gly Pro Gln Leu Leu Ser 675 680 685	2064
ATT ATT CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys 690 695 700	2112
ATT CTA ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn 705 710 715 720	2160
CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro 725 730 735	2208
TCC ACG GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser 740 745 750	2256
CTT CTT GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala 755 760 765	2304
AAA GGA TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu 770 775 780	2352
AGA AAT GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe 785 790 795 800	2400

- 43 -

TCT GGT GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT	2448
Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn	
805 810 815	
GAT TTG CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG	2496
Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met	
820 825 830	
ACT AGT TTC GTT ACC GTA TCA ACG ATT GGA CTA ATT TGG GCG TTA GTA	2544
Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val	
835 840 845	
TCG GGC TGG AAG TTA AGT TTG GTT TGT ATT TCG ATG TTT CCA CTC ATA	2592
Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile	
850 855 860	
ATT ATA TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA	2640
Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr	
865 870 875 880	
GAT TAT AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT	2688
Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile	
885 890 895	
GTC ACT AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT	2736
Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe	
900 905 910	
CAA TTG ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC	2784
Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser	
915 920 925	
AAA CGC GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT	2832
Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile	
930 935 940	
GTC ATG TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT	2880
Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val	
945 950 955 960	
ATG ATT CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG	2928
Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu	
965 970 975	
TTA TTA TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC	2976
Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro	
980 985 990	
GAT ATA AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT	3024
Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu	
995 1000 1005	

- 44 -

GAT GAA AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr 1010 1015 1020	3072
GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1025 1030 1035 1040	3120
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1045 1050 1055	3168
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1060 1065 1070	3216
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu 1075 1080 1085	3264
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly 1090 1095 1100	3312
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1105 1110 1115 1120	3360
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1125 1130 1135	3408
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1140 1145 1150	3456
GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648

- 45 -

ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT	3696
Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val	
1220 1225 1230	
ATA ACG CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT	3744
Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val	
1235 1240 1245	
CTT AAA GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT	3792
Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr	
1250 1255 1260	
AAT AAT CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA	3840
Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser	
1265 1270 1275	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1279 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg
1				5					10					15	
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile
		20					25					30			
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr
		35				40						45			
Gly	Arg	Val	Phe	Asp	Leu	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His
	50					55					60				
Gln	Gly	Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala
	65				70				75				80		
Leu	Gly	Ala	Ala	Ser	Val	Pro	Val	Met	Trp	Leu	Ser	Leu	Thr	Ser	Trp
				85				90					95		
Met	His	Ile	Gly	Glu	Arg	Gln	Gly	Phe	Arg	Ile	Arg	Ser	Gln	Ile	Leu
			100					105					110		
Glu	Ala	Tyr	Leu	Glu	Glu	Lys	Pro	Met	Glu	Trp	Tyr	Asp	Asn	Asn	Glu
		115					120					125			

- 46 -

Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu
 130 135 140
 Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val
 145 150 155 160
 Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu
 165 170 175
 Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val
 180 185 190
 Val Phe Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu
 195 200 205
 Thr Ser Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln
 210 215 220
 Leu Val Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys
 225 230 235 240
 Glu Ile Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe
 245 250 255
 Val Ala Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe
 260 265 270
 Val Gln Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu
 275 280 285
 Asn Ile Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly
 290 295 300
 Ser Thr Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly
 305 310 315 320
 Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys
 325 330 335
 Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr
 340 345 350
 Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser
 355 360 365
 Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala
 370 375 380
 Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr
 385 390 395 400

- 47 -

Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile
 405 410 415
 Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile
 420 425 430
 Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr
 435 440 445
 Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr
 450 455 460
 Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe
 465 470 475 480
 Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser
 485 490 495
 Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp
 500 505 510
 Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val
 515 520 525
 His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys
 530 535 540
 Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp
 545 550 555 560
 Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln
 565 570 575
 Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His
 580 585 590
 Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr
 595 600 605
 Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu
 610 615 620
 Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr
 625 630 635 640
 Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val
 645 650 655
 Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu
 660 665 670

- 48 -

Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser
 675 680 685
 Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys
 690 695 700
 Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn
 705 710 715 720
 Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro
 725 730 735
 Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser
 740 745 750
 Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala
 755 760 765
 Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu
 770 775 780
 Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe
 785 790 795 800
 Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn
 805 810 815
 Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met
 820 825 830
 Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val
 835 840 845
 Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile
 850 855 860
 Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr
 865 870 875 880
 Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile
 885 890 895
 Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe
 900 905 910
 Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser
 915 920 925
 Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile
 930 935 940

- 49 -

Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val
 945 950 955 960
 Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu
 965 970 975
 Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro
 980 985 990
 Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu
 995 1000 1005
 Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr
 1010 1015 1020
 Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile
 1025 1030 1035 1040
 Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala
 1045 1050 1055
 Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu
 1060 1065 1070
 Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu
 1075 1080 1085
 Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly
 1090 1095 1100
 Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser
 1105 1110 1115 1120
 Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn
 1125 1130 1135
 Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp
 1140 1145 1150
 Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln
 1155 1160 1165
 Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala
 1170 1175 1180
 Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu
 1185 1190 1195 1200
 Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ile
 1205 1210 1215

- 50 -

```

Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val
      1220                      1225                      1230

Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val
      1235                      1240                      1245

Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr
      1250                      1255                      1260

Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser
      1265                      1270                      1275

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3786 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic).

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..3786

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1264..1524
- (D) OTHER INFORMATION: /note= "Substituted analogous sequence from human CFTR gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG      48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg
  1           5           10           15

TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA      96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile
      20           25           30

```

- 51 -

GGT ACC GTG GCA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT CAA GGT	144
Gly Thr Val Ala Leu Ser Val Phe Val Ala Asn Gly Ser His Gln Gly	
35 40 45	
TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA CTT GGT	192
Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala Leu Gly	
50 55 60	
GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG ATG CAC	240
Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp Met His	
65 70 75 80	
ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG GAG GCA	288
Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu Glu Ala	
85 90 95	
TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA AAA TTG	336
Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu Lys Leu	
100 105 110	
TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA AGA TCA	384
Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu Arg Ser	
115 120 125	
AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT GCA ATA	432
Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val Ala Ile	
130 135 140	
TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA ACT TTA	480
Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu Thr Leu	
145 150 155 160	
ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG GTG TTT	528
Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val Val Phe	
165 170 175	
TCC AGA ATG ATT CAT GTA TAT TCA GAG AAG GAG AAT TCT GAA ACG AGT	576
Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu Thr Ser	
180 185 190	
AAA GCA GCC CAA TTA CTT ACA TGG TCG ATG AAT GCC GCT CAA TTA GTG	624
Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln Leu Val	
195 200 205	
AGA TTA TAT TGT ACA CAA CGT CTA GAA AGG AAA AAA TTC AAG GAA ATC	672
Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys Glu Ile	
210 215 220 225	
ATA CTA AAT TGT AAC ACT TTC TTC ATC AAG AGT TGC TTT TTT GTT GCT	720
Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe Val Ala	
225 230 235 240	

- 52 -

GCA AAC GCT GGG ATC TTG AGA TTT TTG ACG TTG ACT ATG TTT GTA CAG	768
Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe Val Gln	
245 250 255	
GGA TTC TGG TTT GGT TCC GCA ATG ATC AAA AAG GGC AAG CTG AAC ATT	816
Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu Asn Ile	
260 265 270	
AAC GAT GTA ATC ACT TGC TTC CAT TCA TGC ATT ATG CTG GGC TCG ACT	864
Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly Ser Thr	
275 280 285	
TTA AAT AAT ACA TTA CAC CAA ATA GTT GTT CTT CAA AAA GGC GGA GTG	912
Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly Gly Val	
290 295 300	
GCT ATG GAA AAA ATC ATG ACT CTA TTA AAA GAT GGA TCC AAG CGA AAT	960
Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys Arg Asn	
305 310 315 320	
CCT TTA AAT AAA ACT GTA GCC CAC CAA TTT CCA CTA GAT TAT GCC ACC	1008
Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr Ala Thr	
325 330 335	
AGT GAT CTA ACA TTT GCT AAT GTT TCG TTT TCT TAT CCA AGC AGA CCT	1056
Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser Arg Pro	
340 345 350	
TCG GAA GCA GTT TTA AAG AAC GTT AGT TTA AAT TTC TCT GCA GGA CAA	1104
Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala Gly Gln	
355 360 365	
TTT ACT TTC ATA GTA GGA AAA TCA GGC TCA GGT AAA TCT ACA TTA TCC	1152
Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr Leu Ser	
370 375 380	
AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA TCT ATC	1200
Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile Ser Ile	
385 390 395 400	
AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT GAA AAT	1248
Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile Glu Asn	
405 410 415	
ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC ATT AAA	1296
Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr Ile Lys	
420 425 430	
GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC AGA AGC	1344
Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser	
435 440 445	

- 53 -

GTC	ATC	AAA	GCA	TGC	CAA	CTA	GAA	GAG	GAC	ATC	TCC	AAG	TTT	GCA	GAG	1392
Val	Ile	Lys	Ala	Cys	Gln	Leu	Glu	Glu	Asp	Ile	Ser	Lys	Phe	Ala	Glu	
450						455					460					
AAA	GAC	AAT	ATA	GTT	CTT	GGA	GAA	GGT	GGA	ATC	ACA	CTG	AGT	GGA	GGT	1440
Lys	Asp	Asn	Ile	Val	Leu	Gly	Glu	Gly	Gly	Ile	Thr	Leu	Ser	Gly	Gly	
465					470					475					480	
CAA	CGA	GCA	AGA	ATT	TCT	TTA	GCA	AGA	GCA	GTA	TAC	AAA	GAT	GCT	GAT	1488
Gln	Arg	Ala	Arg	Ile	Ser	Leu	Ala	Arg	Ala	Val	Tyr	Lys	Asp	Ala	Asp	
				485					490					495		
TTG	TAT	TTA	TTA	GAC	TCT	CCT	TTT	GGA	TAC	CTA	GAT	ATT	GTT	CAT	CGC	1536
Leu	Tyr	Leu	Leu	Asp	Ser	Pro	Phe	Gly	Tyr	Leu	Asp	Ile	Val	His	Arg	
			500					505					510			
AAC	CTG	TTG	ATG	AAG	GCA	ATT	AGG	CAT	TGG	AGG	AAA	GGA	AAG	ACT	ACA	1584
Asn	Leu	Leu	Met	Lys	Ala	Ile	Arg	His	Trp	Arg	Lys	Gly	Lys	Thr	Thr	
		515					520					525				
ATC	ATA	TTG	ACG	CAT	GAG	TTG	AGC	CAA	ATT	GAA	TCT	GAT	GAC	TAT	TTA	1632
Ile	Ile	Leu	Thr	His	Glu	Leu	Ser	Gln	Ile	Glu	Ser	Asp	Asp	Tyr	Leu	
	530					535					540					
TAT	TTA	ATG	AAG	GAA	GGT	GAA	GTT	GTT	GAA	AGC	GGC	ACC	CAG	TCT	GAA	1680
Tyr	Leu	Met	Lys	Glu	Gly	Glu	Val	Val	Glu	Ser	Gly	Thr	Gln	Ser	Glu	
				550						555					560	
CTT	CTA	GCC	GAT	CCG	ACC	ACT	ACA	TTT	AGC	ACA	TGG	TAT	CAC	CTA	CAG	1728
Leu	Leu	Ala	Asp	Pro	Thr	Thr	Thr	Phe	Ser	Thr	Trp	Tyr	His	Leu	Gln	
				565					570					575		
AAT	GAC	TAC	TCT	GAT	GCG	AAA	ACT	ATT	GTA	GAT	ACA	GAG	ACT	GAA	GAA	1776
Asn	Asp	Tyr	Ser	Asp	Ala	Lys	Thr	Ile	Val	Asp	Thr	Glu	Thr	Glu	Glu	
			580					585					590			
AAA	TCT	ATA	CAC	ACT	GTG	GAA	AGT	TTT	AAC	TCT	CAA	TTG	GAA	ACA	CCA	1824
Lys	Ser	Ile	His	Thr	Val	Glu	Ser	Phe	Asn	Ser	Gln	Leu	Glu	Thr	Pro	
		595					600					605				
AAA	CTT	GGA	TCA	TGC	TTA	AGT	AAT	CTG	GGA	TAT	GAT	GAG	ACA	GAT	CAG	1872
Lys	Leu	Gly	Ser	Cys	Leu	Ser	Asn	Leu	Gly	Tyr	Asp	Glu	Thr	Asp	Gln	
	610					615					620					
TTG	TCC	TTT	TAC	GAA	GCA	ATC	TAT	CAA	AAA	AGA	TCG	AAC	GTT	AGA	ACA	1920
Leu	Ser	Phe	Tyr	Glu	Ala	Ile	Tyr	Gln	Lys	Arg	Ser	Asn	Val	Arg	Thr	
	625				630					635					640	
AGA	AGG	GTT	AAA	GTT	GAA	GAG	GAA	AAT	ATT	GGG	TAT	GCA	CTA	AAA	CAA	1968
Arg	Arg	Val	Lys	Val	Glu	Glu	Glu	Asn	Ile	Gly	Tyr	Ala	Leu	Lys	Gln	
				645					650					655		

- 54 -

CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC ATT ATT	2016
Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser Ile Ile	
660 665 670	
CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA ATT CTA	2064
Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys Ile Leu	
675 680 685	
ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT CCC GTC	2112
Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn Pro Val	
690 695 700	
TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA TCC ACG	2160
Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro Ser Thr	
705 710 715 720	
GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG CTT CTT	2208
Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser Leu Leu	
725 730 735	
GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT AAA GGA	2256
Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala Lys Gly	
740 745 750	
TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT AGA AAT	2304
Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu Arg Asn	
755 760 765	
GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT TCT GGT	2352
Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe Ser Gly	
770 775 780	
GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT GAT TTG	2400
Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn Asp Leu	
785 790 800	
CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG ACT AGT	2448
Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met Thr Ser	
805 810 815	
TTC GTT ACC GTA TCA ACG ATT GGA CTA ATT TGG GCG TTA GTA TCG GGC	2496
Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val Ser Gly	
820 825 830	
TGG AAG TTA AGT TTG GTT TGT ATT TCG ATG TTT CCA CTC ATA ATT ATA	2544
Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile Ile Ile	
835 840 845	
TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA GAT TAT	2592
Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr Asp Tyr	
850 855 860	

- 55 -

AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT GTC ACT	2640
Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile Val Thr	
865 870 875 880	
AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT CAA TTG	2688
Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe Gln Leu	
885 890 895	
ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC AAA CGC	2736
Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser Lys Arg	
900 905 910	
GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT GTC ATG	2784
Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile Val Met	
915 920 925	
TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT ATG ATT	2832
Cys Ile Gln Ala Ile Ile Tyr Tyr Gly Leu Lys Leu Val Met Ile	
930 935 940	
CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG TTA TTA	2880
His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu Leu	
945 950 955 960	
TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC GAT ATA	2928
Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro Asp Ile	
965 970 975	
AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT GAT GAA	2976
Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu Asp Glu	
980 985 990	
AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA GTG GGA	3024
Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr Val Gly	
995 1000 1005	
ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC GTT TCA	3072
Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile Val Ser	
1010 1015 1020	
ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC TTT GTT	3120
Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val	
1025 1030 1035 1040	
TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA GGT ATC	3168
Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile	
1045 1050 1055	
ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA TTA ACA	3216
Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Thr	
1060 1065 1070	

- 56 -

AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT ACG GAC Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp 1075 1080 1085	3264
GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA GTG GTT Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val 1090 1095 1100	3312
GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC CTA ACT Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr 1105 1110 1115 1120	3360
TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT GCA TTA Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu 1125 1130 1135	3408
AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG GGC TTG Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu 1140 1145 1150	3456
GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG CAA AGG Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg 1155 1160 1165	3504
CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG ATT TTA Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu 1170 1175 1180	3552
GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC ATC AAT Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile Ile Asn 1185 1190 1195 1200	3600
GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT ATA ACG Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr 1205 1210 1215	3648
CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT CTT AAA His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys 1220 1225 1230	3696
GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT AAT AAT Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr Asn Asn 1235 1240 1245	3744
CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser 1250 1255 1260	3786

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- 57 -

(A) LENGTH: 1261 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg
 1           5           10           15
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile
          20           25           30
Gly Thr Val Ala Leu Ser Val Phe Val Ala Asn Gly Ser His Gln Gly
          35           40           45
Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala Leu Gly
 50           55           60
Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp Met His
 65           70           75           80
Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu Glu Ala
          85           90           95
Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu Lys Leu
          100          105          110
Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu Arg Ser
 115          120          125
Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val Ala Ile
 130          135          140
Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu Thr Leu
 145          150          155          160
Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val Val Phe
          165          170          175
Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu Thr Ser
 180          185          190
Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln Leu Val
 195          200          205
Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys Glu Ile
 210          215          220
Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe Val Ala
 225          230          235          240

```

- 58 -

Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe Val Gln
 245 250 255
 Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu Asn Ile
 260 265 270
 Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly Ser Thr
 275 280 285
 Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly Gly Val
 290 295 300
 Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys Arg Asn
 305 310 315 320
 Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr Ala Thr
 325 330 335
 Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser Arg Pro
 340 345 350
 Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala Gly Gln
 355 360 365
 Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr Leu Ser
 370 375 380
 Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile Ser Ile
 385 390 395 400
 Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile Glu Asn
 405 410 415
 Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr Ile Lys
 420 425 430
 Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser
 435 440 445
 Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu
 450 455 460
 Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser Gly Gly
 465 470 475 480
 Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp
 485 490 495
 Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr Leu Asp Ile Val His Arg
 500 505 510

- 59 -

Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys Thr Thr
 515 520 525
 Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp Tyr Leu
 530 535 540
 Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln Ser Glu
 545 550 555 560
 Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His Leu Gln
 565 570 575
 Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr Glu Glu
 580 585 590
 Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu Thr Pro
 595 600 605
 Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr Asp Gln
 610 615 620
 Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val Arg Thr
 625 630 635 640
 Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu Lys Gln
 645 650 655
 Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser Ile Ile
 660 665 670
 Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys Ile Leu
 675 680 685
 Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn Pro Val
 690 695 700
 Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro Ser Thr
 705 710 715 720
 Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser Leu Leu
 725 730 735
 Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala Lys Gly
 740 745 750
 Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu Arg Asn
 755 760 765
 Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe Ser Gly
 770 775 780

- 60 -

Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn Asp Leu
 785 790 795 800
 Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met Thr Ser
 805 810 815
 Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val Ser Gly
 820 825 830
 Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile Ile Ile
 835 840 845
 Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr Asp Tyr
 850 855 860
 Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile Val Thr
 865 870 875 880
 Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe Gln Leu
 885 890 895
 Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser Lys Arg
 900 905 910
 Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile Val Met
 915 920 925
 Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val Met Ile
 930 935 940
 His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu Leu Leu
 945 950 955 960
 Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro Asp Ile
 965 970 975
 Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu Asp Glu
 980 985 990
 Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr Val Gly
 995 1000 1005
 Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile Val Ser
 1010 1015 1020
 Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val
 1025 1030 1035 1040
 Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile
 1045 1050 1055

- 61 -

Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Leu Thr
 1060 1065 1070
 Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp
 1075 1080 1085
 Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val
 1090 1095 1100
 Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr
 1105 1110 1115 1120
 Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu
 1125 1130 1135
 Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu
 1140 1145 1150
 Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg
 1155 1160 1165
 Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu
 1170 1175 1180
 Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ile Ile Asn
 1185 1190 1195 1200
 Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr
 1205 1210 1215
 His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys
 1220 1225 1230
 Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr Asn Asn
 1235 1240 1245
 Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser
 1250 1255 1260

- 62 -

What is claimed is:

1. A method of identifying potential treatments which correct mislocalization and/or misfunction of CFTR resulting from mutations said method comprising: forming a chimeric gene sequence by substituting a portion of a gene sequence which encodes the first nucleotide binding domain of cystic fibrosis conductance regulator with a gene sequence which encodes STE6; said CFTR sequence being a CFTR gene sequence encoding a plurality of amino acids from amino acid 444 to and including amino acid 577, such that introduction of a mutation in the CFTR region inhibits expression of the chimeric STE6 gene product transforming said chimeric gene sequence into a yeast strain with no functional STE6 gene; introducing said potential treatment to said yeast strain; and assaying for the production of diploid yeast colonies following yeast cell mating.
2. The method of claim 1 wherein said mutation is a deletion of phenylalanine at position 508 of the CFTR gene product.
3. The method of claim 1 wherein said CFTR substitute is the gene sequence which encodes from amino acid 494 through 546 of the CFTR gene product (SEQ ID #1).
4. The method of claim 1 wherein said CFTR substitution is the gene sequence which encodes from amino acid 494 up to and including amino acid 558 of the CFTR gene product (SEQ ID #3).

- 63 -

5. The method of claim 1 wherein said CFTR substitution is the gene sequence which encodes from amino acid 494 up to and including 577 of the CFTR gene product (SEQ ID #5).

6. The method of claim 3, 4, or 5 wherein said CFTR substitution includes a mutation which causes cystic fibrosis.

7. A chimeric protein comprising a yeast STE6 protein product having substituted therein an analogous portion of CFTR nucleotide binding domain including a plurality of amino acids from amino acid 443 to amino acid 577.

8. The protein of claim 7 wherein said CFTR sequence includes a mutation which results in cystic fibrosis.

9. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 2.

10. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 4.

11. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 6.

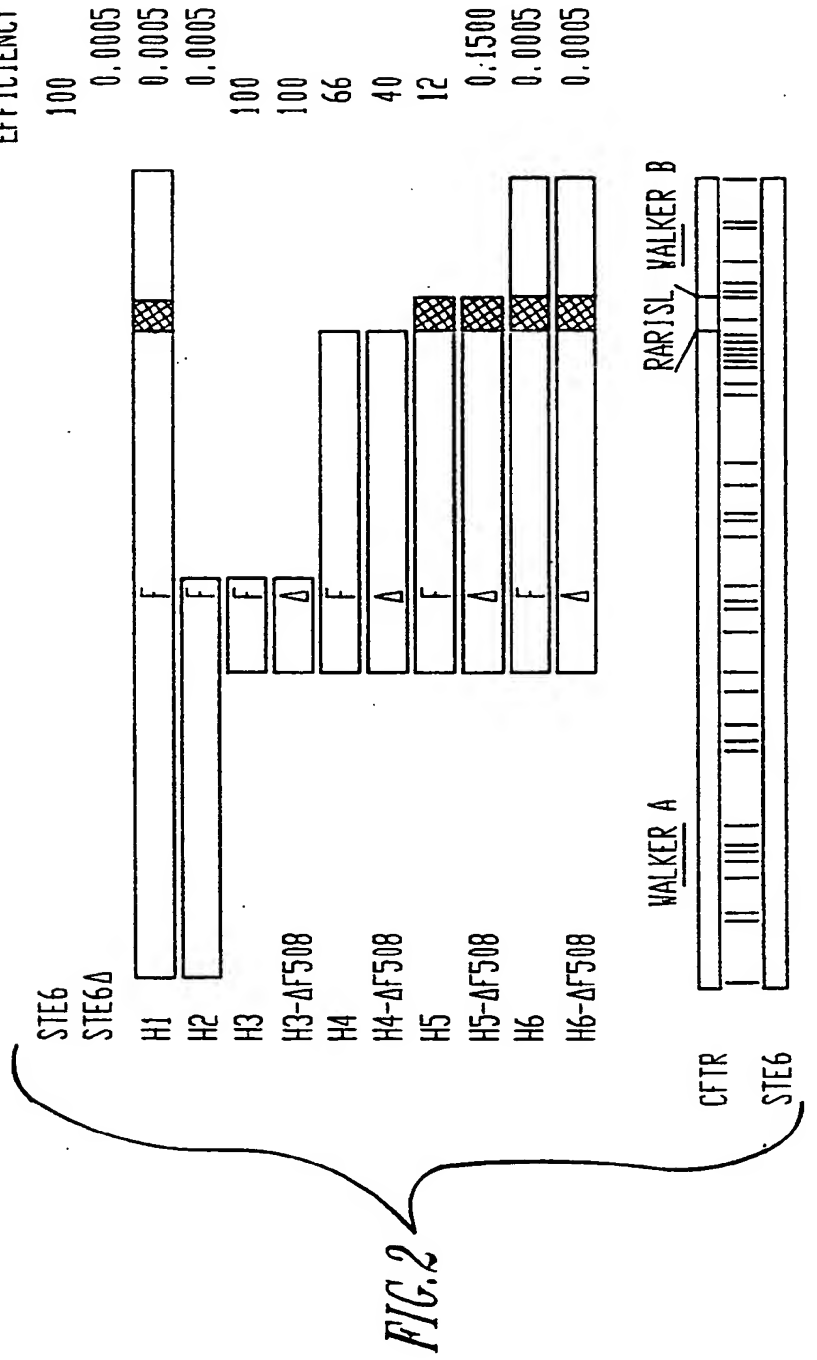
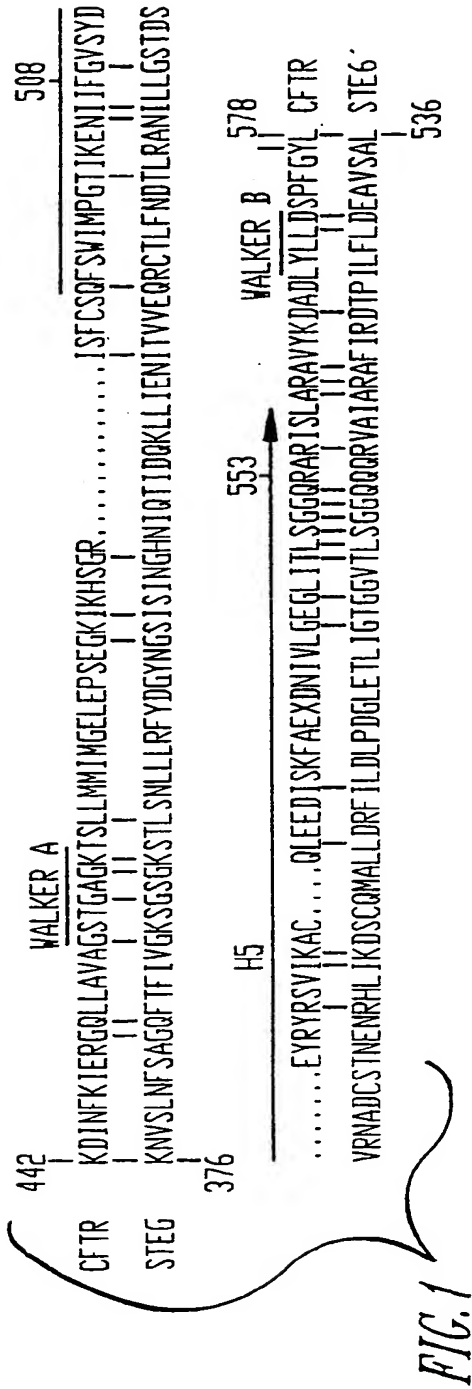
12. A chimeric protein sufficiently homologous to that of claim 7 such that introduction of a mutation in the CFTR portion will prevent transport of a-factor out a yeast cell thereby preventing yeast mating.

- 64 -

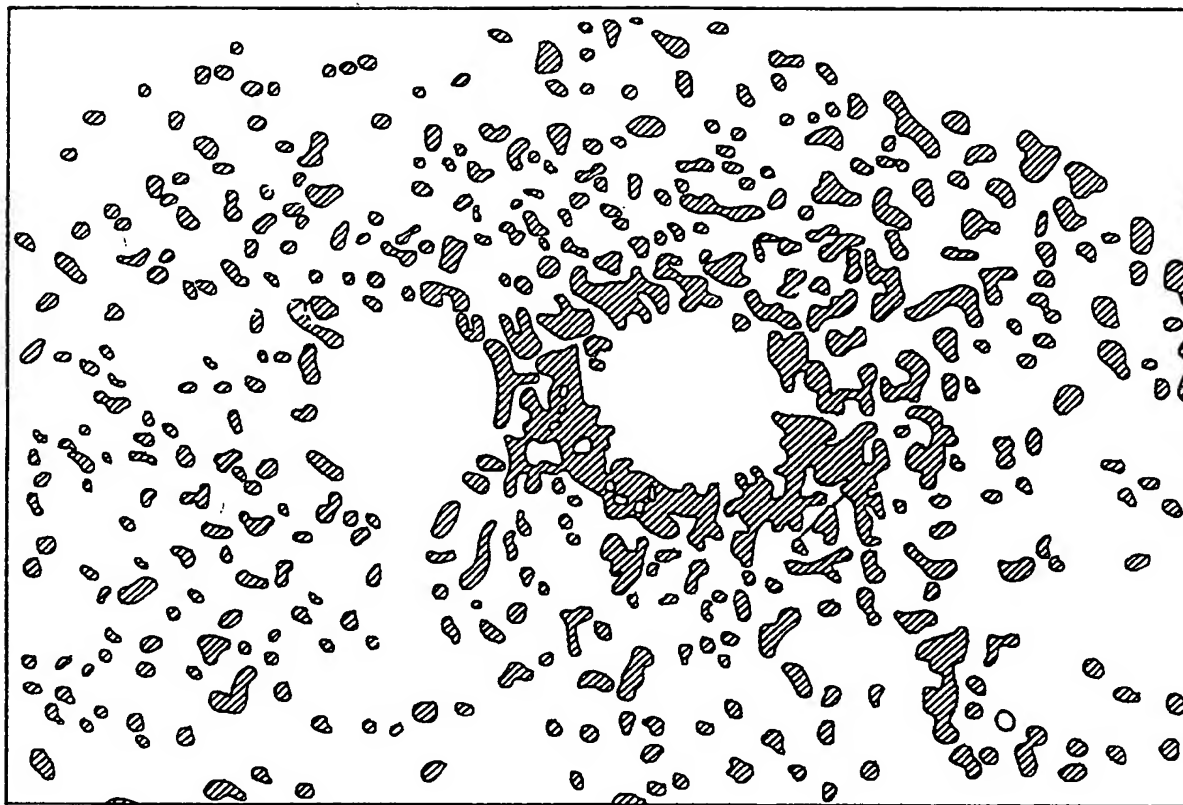
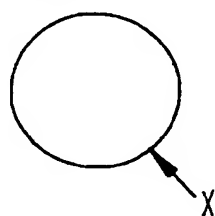
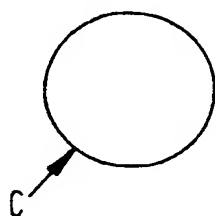
13. A DNA sequence which encodes the protein of claim 7.

14. A host cell transformed with the DNA sequence of claim 13 in a manner allowing expression of the STE6 gene.

15. A method of screening for potential inhibitors of ABC transporters by the use of STE6 chimeras, said method comprising selecting from the class of related proteins consisting essentially of multi-drug resistance (mdr) or P-glycoprotein, the chloroquine resistance transporter (pfmdr) of Plasmodium falciparum, and CFTR; substituting the nucleotide sequence of the STE6 gene with the sequence encoding the analogous portion of said ABC transporter; transforming said chimeric gene sequence into a yeast which is deleted for the STE6 gene; introducing a treatment to said transformed yeast strain; and assaying for the production of yeast diploid colonies following yeast cell mating.



2/23

*FIG. 3*

X=EXTRACT FROM GRINDELIA
C=CONTROL

SELECTIVE MEDIA PLATE WITH DIPLOID COLONIES

3/23

H4 Chimera (Seq. ID No. 1)

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG	48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg	
1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA ACA GGC CTA GTG CCG GCA ATT ACT TCT ATC CTG ACG	144
Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr	
35 40 45	
GGC AGA GTG TTC GAT CTA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT	192
Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His	
50 55 60	
CAA GGT TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA	240
Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala	
65 70 75 80	
CTT GGT GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG	288
Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp	
85 90 95	
ATG CAC ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG	336
Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu	
100 105 110	
GAG GCA TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA	384
Glu Ala Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu	
115 120 125	
AAA TTG TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA	432
Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu	
130 135 140	
AGA TCA AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT	480
Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val	
145 150 155 160	
GCA ATA TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA	528
Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu	
165 170 175	
ACT TTA ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG	576
Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val	
180 185 190	

FIG. 4A

4/23

GTG	TTT	TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	624
Val	Phe	Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	
		195					200					205				
ACG	AGT	AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	672
Thr	Ser	Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	
	210					215					220					
TTA	GTG	AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	720
Leu	Val	Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	
225					230					235					240	
GAA	ATC	ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	768
Glu	Ile	Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	
				245					250					255		
GTT	GCT	GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	816
Val	Ala	Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	
			260					265					270			
GTA	CAG	GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	864
Val	Gln	Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	
		275					280					285				
AAC	ATT	AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	912
Asn	Ile	Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	
	290					295					300					
TCG	ACT	TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	960
Ser	Thr	Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	
305					310					315					320	
GGA	GTG	GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	1008
Gly	Val	Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	
				325					330				335			
CGA	AAT	CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	1056
Arg	Asn	Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	
			340					345					350			
GCC	ACC	AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	1104
Ala	Thr	Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	
		355					360					365				
AGA	CCT	TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	1152
Arg	Pro	Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	
	370				375						380					
GGA	CAA	TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	1200
Gly	Gln	Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	
385					390					395					400	

FIG. 4B

5/23

TTA	TCC	AAC	TTA	TTA	TTA	AGG	TTC	TAC	GAT	GGC	TAT	AAT	GGA	TCG	ATA	1248
Leu	Ser	Asn	Leu	Leu	Leu	Arg	Phe	Tyr	Asp	Gly	Tyr	Asn	Gly	Ser	Ile	
				405					410					415		
TCT	ATC	AAT	GGC	CAC	AAT	ATC	CAA	ACA	ATC	GAC	CAA	AAA	TTG	CTA	ATT	1296
Ser	Ile	Asn	Gly	His	Asn	Ile	Gln	Thr	Ile	Asp	Gln	Lys	Leu	Leu	Ile	
			420					425					430			
GAA	AAT	ATC	ACC	GTC	GTA	GAA	CAG	TTT	TCC	TGG	ATT	ATG	CCT	GGC	ACC	1344
Glu	Asn	Ile	Thr	Val	Val	Glu	Gln	Phe	Ser	Trp	Ile	Met	Pro	Gly	Thr	
		435					440					445				
					STE6			CFTR								
ATT	AAA	GAA	AAT	ATC	ATC	TTT	GGT	GTT	TCC	TAT	GAT	GAA	TAT	AGA	TAC	1392
Ile	Lys	Glu	Asn	Ile	Ile	Phe	Gly	Val	Ser	Tyr	Asp	Glu	Tyr	Arg	Tyr	
	450					455					460					
AGA	AGC	GTC	ATC	AAA	GCA	TGC	CAA	CTA	GAA	GAG	GAC	ATC	TCC	AAG	TTT	1440
Arg	Ser	Val	Ile	Lys	Ala	Cys	Gln	Leu	Glu	Glu	Asp	Ile	Ser	Lys	Phe	
	465				470						475				480	
GCA	GAG	AAA	GAC	AAT	ATA	GTT	CTT	GGA	GAA	GGT	GGA	ATC	ACA	CTG	AGT	1488
Ala	Glu	Lys	Asp	Asn	Ile	Val	Leu	Gly	Glu	Gly	Gly	Ile	Thr	Leu	Ser	
				485					490					495		
													CFTR		STE6	
GGC	GGG	CAA	CAA	CAA	AGA	GTT	GCT	ATA	GCA	CGT	GCA	TTC	ATC	AGA	GAT	1536
Gly	Gly	Gln	Gln	Gln	Arg	Val	Ala	Ile	Ala	Arg	Ala	Phe	Ile	Arg	Asp	
			500					505					510			
ACT	CCA	ATA	TTA	TTC	TTA	GAC	GAA	GCT	GTA	TCG	GCT	CTA	GAT	ATT	GTT	1584
Thr	Pro	Ile	Leu	Phe	Leu	Asp	Glu	Ala	Val	Ser	Ala	Leu	Asp	Ile	Val	
		515					520					525				
CAT	CGC	AAC	CTG	TTG	ATG	AAG	GCA	ATT	AGG	CAT	TGG	AGG	AAA	GGA	AAG	1632
His	Arg	Asn	Leu	Leu	Met	Lys	Ala	Ile	Arg	His	Trp	Arg	Lys	Gly	Lys	
	530					535					540					
ACT	ACA	ATC	ATA	TTG	ACG	CAT	GAG	TTG	AGC	CAA	ATT	GAA	TCT	GAT	GAC	1680
Thr	Thr	Ile	Ile	Leu	Thr	His	Glu	Leu	Ser	Gln	Ile	Glu	Ser	Asp	Asp	
	545				550					555				560		
TAT	TTA	TAT	TTA	ATG	AAG	GAA	GGT	GAA	GTT	GTT	GAA	AGC	GGC	ACC	CAG	1728
Tyr	Leu	Tyr	Leu	Met	Lys	Glu	Gly	Glu	Val	Val	Glu	Ser	Gly	Thr	Gln	
				565					570					575		
TCT	GAA	CTT	CTA	GCC	GAT	CCG	ACC	ACT	ACA	TTT	AGC	ACA	TGG	TAT	CAC	1776
Ser	Glu	Leu	Leu	Ala	Asp	Pro	Thr	Thr	Thr	Phe	Ser	Thr	Trp	Tyr	His	
			580					585					590			
CTA	CAG	AAT	GAC	TAC	TCT	GAT	GCG	AAA	ACT	ATT	GTA	GAT	ACA	GAG	ACT	1824
Leu	Gln	Asn	Asp	Tyr	Ser	Asp	Ala	Lys	Thr	Ile	Val	Asp	Thr	Glu	Thr	
		595					600					605				

FIG. 4C

6/23

GAA Glu 610	GAA Glu 610	AAA Lys	TCT Ser	ATA Ile	CAC His	ACT Thr 615	GTG Val	GAA Glu	AGT Ser	TTT Phe	AAC Asn 620	TCT Ser	CAA Gln	TTG Leu	GAA Glu	1872
ACA Thr 625	CCA Pro	AAA Lys	CTT Leu	GGA Gly 630	TCA Cys	TGC Leu	TTA Ser	AGT Asn 635	AAT Leu	CTG Gly 635	GGA Tyr	TAT Asp	GAT Glu	GAG Thr 640	ACA Thr	1920
GAT Asp	CAG Gln	TTG Leu	TCC Ser	TTT Phe 645	TAC Tyr	GAA Glu	GCA Ala	ATC Ile	TAT Tyr 650	CAA Gln	AAA Lys	AGA Arg	TCG Ser	AAC Asn 655	GTT Val	1968
AGA Arg	ACA Thr	AGA Arg	AGG Arg 660	GTT Val	AAA Lys	GTT Val	GAA Glu	GAG Glu	GAA Glu	AAT Asn	ATT Ile	GGG Gly	TAT Tyr 670	GCA Ala	CTA Leu	2016
AAA Lys	CAA Gln 675	CAA Gln	AAG Lys	AAC Asn	ACC Thr	GAA Glu	AGT Ser 680	TCA Ser	ACA Thr	GGG Gly	CCA Pro	CAA Gln 685	CTT Leu	CTG Leu	AGC Ser	2064
ATT Ile 690	ATT Ile	CAG Gln	ATT Ile	ATC Ile	AAA Lys	AGA Arg	ATG Met 695	ATT Ile	AAA Lys	AGC Ser	ATA Ile 700	AGA Arg	TAC Tyr	AAA Lys	AAA Lys	2112
ATT Ile 705	CTA Leu	ATC Ile	TTG Leu	GGA Gly	CTG Leu 710	CTA Leu	TGT Cys	TCT Ser	CTT Leu	ATC Ile 715	GCA Ala	GGC Gly	GCC Ala	ACA Thr	AAT Asn 720	2160
CCC Pro	GTC Val	TTT Phe	TCA Ser	TAC Tyr 725	ACA Thr	TTC Phe	AGT Ser	TTC Phe	TTA Leu 730	CTA Leu	GAA Glu	GGA Gly	ATT Ile	GTC Val 735	CCA Pro	2208
TCC Ser	ACG Thr	GAT Asp	GGA Gly 740	AAA Lys	ACT Thr	GGC Gly	TCT Ser	TCA Ser 745	CAT His	TAT Tyr	TTG Leu	GCG Ala	AAA Lys 750	TGG Trp	TCG Ser	2256
CTT Leu	CTT Leu	GTT Val 755	CTT Leu	GGT Gly	GTG Val	GCT Ala	GCG Ala 760	GCA Ala	GAT Asp	GGT Gly	ATT Ile	TTC Phe 765	AAT Asn	TTT Phe	GCT Ala	2304
AAA Lys 770	GGA Gly	TTC Phe	CTA Leu	TTA Leu	GAT Asp	TGC Cys 775	TGC Cys	AGT Ser	GAA Glu	TAC Tyr	TGG Trp 780	GTT Val	ATG Met	GAT Asp	CTT Leu	2352
AGA Arg 785	AAT Asn	GAA Glu	GTT Val	ATG Met	GAA Glu 790	AAA Lys	CTG Leu	ACG Thr	AGA Arg	AAG Lys 795	AAT Asn	ATG Met	GAC Asp	TGG Trp	TTT Phe 800	2400
TCT Ser	GGT Gly	GAA Glu	AAC Asn	AAC Asn	AAG Lys	GCT Ala	TCT Ser	GAA Glu	ATT Ile 810	TCT Ser	GCT Ala	CTA Leu	GTC Val	TTG Leu 815	AAT Asn	2448

FIG. 4D

7/23

GAT	TTG	CGA	GAT	TTG	AGG	TCT	TTG	GTC	TCT	GAA	TTT	TTG	AGT	GCA	ATG	2496
Asp	Leu	Arg	Asp	Leu	Arg	Ser	Leu	Val	Ser	Glu	Phe	Leu	Ser	Ala	Met	
			820					825					830			
ACT	AGT	TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	2544
Thr	Ser	Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	
		835					840					845				
TCG	GGC	TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	2592
Ser	Gly	Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	
	850					855					860					
ATT	ATA	TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	2640
Ile	Ile	Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	
865					870					875					880	
GAT	TAT	AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	2688
Asp	Tyr	Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	
				885					890					895		
GTC	ACT	AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	2736
Val	Thr	Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	
			900					905					910			
CAA	TTG	ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	2784
Gln	Leu	Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	
		915					920					925				
AAA	CGC	GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	2832
Lys	Arg	Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	
	930					935					940					
GTC	ATG	TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	2880
Val	Met	Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr	Tyr	Gly	Leu	Lys	Leu	Val	
945				950						955					960	
ATG	ATT	CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	2928
Met	Ile	His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	
				965					970					975		
TTA	TTA	TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	2976
Leu	Leu	Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	
			980					985					990			
GAT	ATA	AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	3024
Asp	Ile	Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	
		995					1000					1005				
GAT	GAA	AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	3072
Asp	Glu	Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	
	1010					1015					1020					

FIG. 4E

8/23

GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1025 1030 1035 1040	3120
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1045 1050 1055	3168
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1060 1065 1070	3216
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu 1075 1080 1085	3264
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly 1090 1095 1100	3312
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1105 1110 1115 1120	3360
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1125 1130 1135	3408
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1140 1145 1150	3456
GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648
ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val 1220 1225 1230	3696

FIG. 4F

9/23

ATA	ACG	CAT	AGT	GAA	CAA	ATG	ATG	AGG	TCT	TGT	AAC	TCG	ATT	GCA	GTT	3744
Ile	Thr	His	Ser	Glu	Gln	Met	Met	Arg	Ser	Cys	Asn	Ser	Ile	Ala	Val	
			1235					1240					1245			
CTT	AAA	GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	3792
Leu	Lys	Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	
		1250					1255					1260				
AAT	AAT	CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA	3840
Asn	Asn	Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*	
1265						1270				1275					1280	

FIG. 4G

10/23

H5 Chimera (Seq. ID No. 3)

ATG	AAC	TTT	TTA	AGT	TTT	AAG	ACT	ACA	AAA	CAC	TAT	CAC	ATT	TTC	AGG	48
Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg	
1				5					10					15		
TAC	GTG	AAC	ATA	CGG	AAT	GAC	TAC	AGG	CTG	TTA	ATG	ATA	ATG	ATA	ATA	96
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile	
			20					25					30			
GGT	ACC	GTG	GCA	ACA	GGC	CTA	GTG	CCG	GCA	ATT	ACT	TCT	ATC	CTG	ACG	144
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr	
		35				40						45				
GGC	AGA	GTG	TTC	GAT	CTA	CTA	TCA	GTT	TTC	GTG	GCT	AAT	GGG	TCA	CAT	192
Gly	Arg	Val	Phe	Asp	Leu	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His	
	50					55					60					
CAA	GGT	TTG	TAT	TCC	CAA	CTA	GTA	CAG	AGG	TCA	ATG	GCA	GTA	ATG	GCA	240
Gln	Gly	Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala	
65					70				75						80	
CTT	GGT	GCG	GCT	TCT	GTG	CCA	GTA	ATG	TGG	CTT	TCT	CTA	ACA	AGT	TGG	288
Leu	Gly	Ala	Ala	Ser	Val	Pro	Val	Met	Trp	Leu	Ser	Leu	Thr	Ser	Trp	
				85					90					95		
ATG	CAC	ATC	GGC	GAG	AGA	CAA	GGC	TTT	AGA	ATA	CGG	TCA	CAG	ATA	TTG	336
Met	His	Ile	Gly	Glu	Arg	Gln	Gly	Phe	Arg	Ile	Arg	Ser	Gln	Ile	Leu	
			100				105						110			
GAG	GCA	TAT	TTG	GAG	GAA	AAG	CCA	ATG	GAA	TGG	TAC	GAC	AAT	AAT	GAA	384
Glu	Ala	Tyr	Leu	Glu	Glu	Lys	Pro	Met	Glu	Trp	Tyr	Asp	Asn	Asn	Glu	
		115					120					125				
AAA	TTG	TTA	GGA	GAT	TTT	ACT	CAA	ATC	AAC	AGA	TGT	GTG	GAA	GAG	CTA	432
Lys	Leu	Leu	Gly	Asp	Phe	Thr	Gln	Ile	Asn	Arg	Cys	Val	Glu	Glu	Leu	
	130					135					140					
AGA	TCA	AGC	TCC	GCA	GAG	GCA	TCA	GCC	ATA	ACT	TTC	CAG	AAT	TTA	GTT	480
Arg	Ser	Ser	Ser	Ala	Glu	Ala	Ser	Ala	Ile	Thr	Phe	Gln	Asn	Leu	Val	
145					150					155					160	
GCA	ATA	TGT	GCG	CTT	CTG	GGG	ACG	TCA	TTC	TAC	TAT	TCT	TGG	TCA	TTA	528
Ala	Ile	Cys	Ala	Leu	Leu	Gly	Thr	Ser	Phe	Tyr	Tyr	Ser	Trp	Ser	Leu	
				165					170					175		
ACT	TTA	ATT	ATT	CTT	TGC	AGC	TCT	CCA	ATA	ATC	ACA	TTT	TTT	GCA	GTG	576
Thr	Leu	Ile	Ile	Leu	Cys	Ser	Ser	Pro	Ile	Ile	Thr	Phe	Phe	Ala	Val	
			180					185					190			

FIG. 5A

11/23

GTG	TTT	TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	624
Val	Phe	Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	
		195					200					205				
ACG	AGT	AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	672
Thr	Ser	Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	
	210					215					220					
TTA	GTG	AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	720
Leu	Val	Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	
225					230					235					240	
GAA	ATC	ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	768
Glu	Ile	Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	
				245					250					255		
GTT	GCT	GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	816
Val	Ala	Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	
			260					265					270			
GTA	CAG	GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	864
Val	Gln	Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	
		275					280					285				
AAC	ATT	AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	912
Asn	Ile	Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	
	290					295					300					
TCG	ACT	TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	960
Ser	Thr	Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	
305					310					315					320	
GGA	GTG	GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	1008
Gly	Val	Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	
				325					330				335			
CGA	AAT	CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	1056
Arg	Asn	Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	
			340					345					350			
GCC	ACC	AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	1104
Ala	Thr	Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	
		355					360					365				
AGA	CCT	TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	1152
Arg	Pro	Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	
	370					375					380					
GGA	CAA	TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	1200
Gly	Gln	Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	
385					390					395					400	

FIG.5B

12/23

TTA TCC AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA	1248
Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile	
405 410 415	
TCT ATC AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT	1296
Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile	
420 425 430	
GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC	1344
Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr	
435 440 445	
STE6 CFTR	
ATT AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC	1392
Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr	
450 455 460	
AGA AGC GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT	1440
Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe	
465 470 475 480	
GCA GAG AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT	1488
Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser	
485 490 495	
GGA GGT CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA TTC ATC AGA GAT	1536
Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp	
500 505 510	
CFTR STE6	
ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT	1584
Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val	
515 520 525	
CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG	1632
His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys	
530 535 540	
ACT ACA ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC	1680
Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp	
545 550 555 560	
TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG	1728
Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln	
565 570 575	
TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC	1776
Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His	
580 585 590	
CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT	1824
Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr	
595 600 605	

FIG.5C

13/23

GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu 610 615 620	1872
ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr 625 630 635 640	1920
GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val 645 650 655	1968
AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA Arg Thr Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu 660 665 670	2016
AAA CAA CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC Lys Gln Gln Lys Asn Thr Glu Ser Thr Gly Pro Gln Leu Leu Ser 675 680 685	2064
ATT ATT CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys 690 695 700	2112
ATT CTA ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn 705 710 715 720	2160
CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro 725 730 735	2208
TCC ACG GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser 740 745 750	2256
CTT CTT GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala 755 760 765	2304
AAA GGA TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu 770 775 780	2352
AGA AAT GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe 785 790 795 800	2400
TCT GGT GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn 805 810 815	2448

FIG.5D

14/23

GAT	TTG	CGA	GAT	TTG	AGG	TCT	TTG	GTC	TCT	GAA	TTT	TTG	AGT	GCA	ATG	2496
Asp	Leu	Arg	Asp	Leu	Arg	Ser	Leu	Val	Ser	Glu	Phe	Leu	Ser	Ala	Met	
			820					825					830			
ACT	AGT	TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	2544
Thr	Ser	Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	
		835					840					845				
TCG	GGC	TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	2592
Ser	Gly	Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	
	850					855					860					
ATT	ATA	TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	2640
Ile	Ile	Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	
865					870					875					880	
GAT	TAT	AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	2688
Asp	Tyr	Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	
				885					890					895		
GTC	ACT	AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	2736
Val	Thr	Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	
			900					905					910			
CAA	TTG	ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	2784
Gln	Leu	Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	
		915					920					925				
AAA	CGC	GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	2832
Lys	Arg	Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	
	930					935					940					
GTC	ATG	TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	2880
Val	Met	Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr	Tyr	Gly	Leu	Lys	Leu	Val	
945					950					955					960	
ATG	ATT	CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	2928
Met	Ile	His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	
				965					970					975		
TTA	TTA	TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	2976
Leu	Leu	Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	
			980					985					990			
GAT	ATA	AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	3024
Asp	Ile	Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	
		995					1000					1005				
GAT	GAA	AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	3072
Asp	Glu	Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	
	1010					1015						1020				

FIG.5E

15/23

GTG	GGA	ATA	GCT	GGT	CAC	ACC	TAC	CAT	GGC	AAA	GAA	AAA	AAA	CCA	ATC	3120
Val	Gly	Ile	Ala	Gly	His	Thr	Tyr	His	Gly	Lys	Glu	Lys	Lys	Pro	Ile	
1025					1030					1035					1040	
GTT	TCA	ATT	CAA	AAT	TTG	ACA	TTT	GCC	TAT	CCA	TCT	GCA	CCT	ACC	GCC	3168
Val	Ser	Ile	Gln	Asn	Leu	Thr	Phe	Ala	Tyr	Pro	Ser	Ala	Pro	Thr	Ala	
				1045					1050					1055		
TTT	GTT	TAC	AAA	AAC	ATG	AAT	TTT	GAC	ATG	TTT	TGC	GGA	CAG	ACG	TTA	3216
Phe	Val	Tyr	Lys	Asn	Met	Asn	Phe	Asp	Met	Phe	Cys	Gly	Gln	Thr	Leu	
			1060					1065					1070			
GGT	ATC	ATT	GGT	GAA	TCA	GGC	ACA	GGA	AAG	TCT	ACA	CTT	GTG	CTT	TTA	3264
Gly	Ile	Ile	Gly	Glu	Ser	Gly	Thr	Gly	Lys	Ser	Thr	Leu	Val	Leu	Leu	
		1075					1080					1085				
TTA	ACA	AAA	CTT	TAT	AAT	TGT	GAA	GTA	GGC	AAA	ATT	AAA	ATA	GAC	GGT	3312
Leu	Thr	Lys	Leu	Tyr	Asn	Cys	Glu	Val	Gly	Lys	Ile	Lys	Ile	Asp	Gly	
	1090					1095					1100					
ACG	GAC	GTA	AAT	GAC	TGG	AAT	TTG	ACA	AGT	TTA	AGA	AAA	GAA	ATT	TCA	3360
Thr	Asp	Val	Asn	Asp	Trp	Asn	Leu	Thr	Ser	Leu	Arg	Lys	Glu	Ile	Ser	
1105					1110					1115					1120	
GTG	GTT	GAG	CAA	AAA	CCT	TTA	TTA	TTC	AAT	GGA	ACC	ATC	AGA	GAT	AAC	3408
Val	Val	Glu	Gln	Lys	Pro	Leu	Leu	Phe	Asn	Gly	Thr	Ile	Arg	Asp	Asn	
				1125					1130					1135		
CTA	ACT	TAT	GGT	TTA	CAA	GAT	GAA	ATA	CTT	GAA	ATT	GAA	ATG	TAT	GAT	3456
Leu	Thr	Tyr	Gly	Leu	Gln	Asp	Glu	Ile	Leu	Glu	Ile	Glu	Met	Tyr	Asp	
			1140				1145						1150			
GCA	TTA	AAA	TAC	GTA	GGA	ATC	CAT	GAC	TTT	GTA	ATT	TCA	TCA	CCT	CAG	3504
Ala	Leu	Lys	Tyr	Val	Gly	Ile	His	Asp	Phe	Val	Ile	Ser	Ser	Pro	Gln	
		1155				1160						1165				
GGC	TTG	GAT	ACA	CGT	ATT	GAT	ACA	ACT	TTA	CTG	TCA	GGT	GGA	CAA	GCG	3552
Gly	Leu	Asp	Thr	Arg	Ile	Asp	Thr	Thr	Leu	Leu	Ser	Gly	Gly	Gln	Ala	
	1170				1175						1180					
CAA	AGG	CTT	TGC	ATA	GCC	AGA	GCA	CTT	CTG	AGA	AAA	TCA	AAA	ATT	CTG	3600
Gln	Arg	Leu	Cys	Ile	Ala	Arg	Ala	Leu	Leu	Arg	Lys	Ser	Lys	Ile	Leu	
1185					1190					1195					1200	
ATT	TTA	GAT	GAG	TGT	ACT	TCA	GCC	TTG	GAT	TCT	GTC	AGC	TCC	TCT	ATC	3648
Ile	Leu	Asp	Glu	Cys	Thr	Ser	Ala	Leu	Asp	Ser	Val	Ser	Ser	Ser	Ile	
				1205					1210					1215		
ATC	AAT	GAG	ATC	GTC	AAA	AAA	GGT	CCA	CCT	GCT	CTA	CTA	ACA	ATG	GTT	3696
Ile	Asn	Glu	Ile	Val	Lys	Lys	Gly	Pro	Pro	Ala	Leu	Leu	Thr	Met	Val	
			1220					1225					1230			

FIG. 5F

16/23

ATA	ACG	CAT	AGT	GAA	CAA	ATG	ATG	AGG	TCT	TGT	AAC	TCG	ATT	GCA	GTT	3744
Ile	Thr	His	Ser	Glu	Gln	Met	Met	Arg	Ser	Cys	Asn	Ser	Ile	Ala	Val	
		1235						1240					1245			
CTT	AAA	GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	3792
Leu	Lys	Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	
		1250						1255					1260			
AAT	AAT	CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA	3840
Asn	Asn	Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*	
		1265						1270					1275			1280

FIG.5G

17/23

H6 Chimera (Seq. ID No. 5)

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG	48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg	
1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT CAA GGT	144
Gly Thr Val Ala Leu Ser Val Phe Val Ala Asn Gly Ser His Gln Gly	
35 40 45	
TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA CTT GGT	192
Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala Leu Gly	
50 55 60	
GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG ATG CAC	240
Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp Met His	
65 70 75 80	
ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG GAG GCA	288
Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu Glu Ala	
85 90 95	
TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA AAA TTG	336
Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu Lys Leu	
100 105 110	
TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA AGA TCA	384
Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu Arg Ser	
115 120 125	
AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT GCA ATA	432
Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val Ala Ile	
130 135 140	
TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA ACT TTA	480
Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu Thr Leu	
145 150 155 160	
ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG GTG TTT	528
Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val Val Phe	
165 170 175	
TCC AGA ATG ATT CAT GTA TAT TCA GAG AAG GAG AAT TCT GAA ACG AGT	576
Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu Thr Ser	
180 185 190	

FIG. 6A

18/23

AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	TTA	GTG	624
Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	Leu	Val	
		195					200					205				
AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	GAA	ATC	672
Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	Glu	Ile	
	210					215					220					
ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	GTT	GCT	720
Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	Val	Ala	
	225				230					235				240		
GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	GTA	CAG	768
Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	Val	Gln	
			245					250						255		
GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	AAC	ATT	816
Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	Asn	Ile	
			260					265					270			
AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	TCG	ACT	864
Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	Ser	Thr	
		275					280					285				
TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	GGA	GTG	912
Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	Gly	Val	
	290					295					300					
GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	CGA	AAT	960
Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	Arg	Asn	
	305				310					315					320	
CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	GCC	ACC	1008
Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	Ala	Thr	
			325						330					335		
AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	AGA	CCT	1056
Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	Arg	Pro	
			340					345					350			
TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	GGA	CAA	1104
Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	Gly	Gln	
		355					360					365				
TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	TTA	TCC	1152
Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	Leu	Ser	
	370					375					380					
AAC	TTA	TTA	TTA	AGG	TTC	TAC	GAT	GGC	TAT	AAT	GGA	TCG	ATA	TCT	ATC	1200
Asn	Leu	Leu	Leu	Arg	Phe	Tyr	Asp	Gly	Tyr	Asn	Gly	Ser	Ile	Ser	Ile	
					390					395					400	

FIG. 6B

19/23

AAT	GGC	CAC	AAT	ATC	CAA	ACA	ATC	GAC	CAA	AAA	TTG	CTA	ATT	GAA	AAT	1248
Asn	Gly	His	Asn	Ile	Gln	Thr	Ile	Asp	Gln	Lys	Leu	Leu	Ile	Glu	Asn	
				405					410					415		
ATC	ACC	GTC	GTA	GAA	CAG	TTT	TCC	TGG	ATT	ATG	CCT	GGC	ACC	ATT	AAA	1296
Ile	Thr	Val	Val	Glu	Gln	Phe	Ser	Trp	Ile	Met	Pro	Gly	Thr	Ile	Lys	
			420					425					430			
			STE6			CFTR										
GAA	AAT	ATC	ATC	TTT	GGT	GTT	TCC	TAT	GAT	GAA	TAT	AGA	TAC	AGA	AGC	1344
Glu	Asn	Ile	Ile	Phe	Gly	Val	Ser	Tyr	Asp	Glu	Tyr	Arg	Tyr	Arg	Ser	
		435					440					445				
GTC	ATC	AAA	GCA	TGC	CAA	CTA	GAA	GAG	GAC	ATC	TCC	AAG	TTT	GCA	GAG	1392
Val	Ile	Lys	Ala	Cys	Gln	Leu	Glu	Glu	Asp	Ile	Ser	Lys	Phe	Ala	Glu	
		450				455					460					
AAA	GAC	AAT	ATA	GTT	CTT	GGA	GAA	GGT	GGA	ATC	ACA	CTG	AGT	GGA	GGT	1440
Lys	Asp	Asn	Ile	Val	Leu	Gly	Glu	Gly	Gly	Ile	Thr	Leu	Ser	Gly	Gly	
					470					475					480	
CAA	CGA	GCA	AGA	ATT	TCT	TTA	GCA	AGA	GCA	GTA	TAC	AAA	GAT	GCT	GAT	1488
Gln	Arg	Ala	Arg	Ile	Ser	Leu	Ala	Arg	Ala	Val	Tyr	Lys	Asp	Ala	Asp	
				485					490					495		
TTG	TAT	TTA	TTA	GAC	TCT	CCT	TTT	GGA	TAC	CTA	GAT	ATT	GTT	CAT	CGC	1536
Leu	Tyr	Leu	Leu	Asp	Ser	Pro	Phe	Gly	Tyr	Leu	Asp	Ile	Val	His	Arg	
			500					505					510			
									CFTR			STE6				
AAC	CTG	TTG	ATG	AAG	GCA	ATT	AGG	CAT	TGG	AGG	AAA	GGA	AAG	ACT	ACA	1584
Asn	Leu	Leu	Met	Lys	Ala	Ile	Arg	His	Trp	Arg	Lys	Gly	Lys	Thr	Thr	
			515				520					525				
ATC	ATA	TTG	ACG	CAT	GAG	TTG	AGC	CAA	ATT	GAA	TCT	GAT	GAC	TAT	TTA	1632
Ile	Ile	Leu	Thr	His	Glu	Leu	Ser	Gln	Ile	Glu	Ser	Asp	Asp	Tyr	Leu	
		530				535					540					
TAT	TTA	ATG	AAG	GAA	GGT	GAA	GTT	GTT	GAA	AGC	GGC	ACC	CAG	TCT	GAA	1680
Tyr	Leu	Met	Lys	Glu	Gly	Glu	Val	Val	Glu	Ser	Gly	Thr	Gln	Ser	Glu	
		545			550					555					560	
CTT	CTA	GCC	GAT	CCG	ACC	ACT	ACA	TTT	AGC	ACA	TGG	TAT	CAC	CTA	CAG	1728
Leu	Leu	Ala	Asp	Pro	Thr	Thr	Thr	Phe	Ser	Thr	Trp	Tyr	His	Leu	Gln	
				565					570					575		
AAT	GAC	TAC	TCT	GAT	GCG	AAA	ACT	ATT	GTA	GAT	ACA	GAG	ACT	GAA	GAA	1776
Asn	Asp	Tyr	Ser	Asp	Ala	Lys	Thr	Ile	Val	Asp	Thr	Glu	Thr	Glu	Glu	
			580					585					590			
AAA	TCT	ATA	CAC	ACT	GTG	GAA	AGT	TTT	AAC	TCT	CAA	TTG	GAA	ACA	CCA	1824
Lys	Ser	Ile	His	Thr	Val	Glu	Ser	Phe	Asn	Ser	Gln	Leu	Glu	Thr	Pro	
		595					600					605				

FIG. 6C

20/23

AAA	CTT	GGA	TCA	TGC	TTA	AGT	AAT	CTG	GGA	TAT	GAT	GAG	ACA	GAT	CAG	1872
Lys	Leu	Gly	Ser	Cys	Leu	Ser	Asn	Leu	Gly	Tyr	Asp	Glu	Thr	Asp	Gln	
610						615					620					
TTG	TCC	TTT	TAC	GAA	GCA	ATC	TAT	CAA	AAA	AGA	TCG	AAC	GTT	AGA	ACA	1920
Leu	Ser	Phe	Tyr	Glu	Ala	Ile	Tyr	Gln	Lys	Arg	Ser	Asn	Val	Arg	Thr	
625					630					635					640	
AGA	AGG	GTT	AAA	GTT	GAA	GAG	GAA	AAT	ATT	GGG	TAT	GCA	CTA	AAA	CAA	1968
Arg	Arg	Val	Lys	Val	Glu	Glu	Glu	Asn	Ile	Gly	Tyr	Ala	Leu	Lys	Gln	
				645					650					655		
CAA	AAG	AAC	ACC	GAA	AGT	TCA	ACA	GGG	CCA	CAA	CTT	CTG	AGC	ATT	ATT	2016
Gln	Lys	Asn	Thr	Glu	Ser	Ser	Thr	Gly	Pro	Gln	Leu	Leu	Ser	Ile	Ile	
			660					665					670			
CAG	ATT	ATC	AAA	AGA	ATG	ATT	AAA	AGC	ATA	AGA	TAC	AAA	AAA	ATT	CTA	2064
Gln	Ile	Ile	Lys	Arg	Met	Ile	Lys	Ser	Ile	Arg	Tyr	Lys	Lys	Ile	Leu	
		675					680					685				
ATC	TTG	GGA	CTG	CTA	TGT	TCT	CTT	ATC	GCA	GGC	GCC	ACA	AAT	CCC	GTC	2112
Ile	Leu	Gly	Leu	Leu	Cys	Ser	Leu	Ile	Ala	Gly	Ala	Thr	Asn	Pro	Val	
	690					695					700					
TTT	TCA	TAC	ACA	TTC	AGT	TTC	TTA	CTA	GAA	GGA	ATT	GTC	CCA	TCC	ACG	2160
Phe	Ser	Tyr	Thr	Phe	Ser	Phe	Leu	Leu	Glu	Gly	Ile	Val	Pro	Ser	Thr	
705					710					715					720	
GAT	GGA	AAA	ACT	GGC	TCT	TCA	CAT	TAT	TTG	GCG	AAA	TGG	TCG	CTT	CTT	2208
Asp	Gly	Lys	Thr	Gly	Ser	Ser	His	Tyr	Leu	Ala	Lys	Trp	Ser	Leu	Leu	
				725					730					735		
GTT	CTT	GGT	GTG	GCT	GCG	GCA	GAT	GGT	ATT	TTC	AAT	TTT	GCT	AAA	GGA	2256
Val	Leu	Gly	Val	Ala	Ala	Ala	Asp	Gly	Ile	Phe	Asn	Phe	Ala	Lys	Gly	
			740					745					750			
TTC	CTA	TTA	GAT	TGC	TGC	AGT	GAA	TAC	TGG	GTT	ATG	GAT	CTT	AGA	AAT	2304
Phe	Leu	Leu	Asp	Cys	Cys	Ser	Glu	Tyr	Trp	Val	Met	Asp	Leu	Arg	Asn	
		755				760						765				
GAA	GTT	ATG	GAA	AAA	CTG	ACG	AGA	AAG	AAT	ATG	GAC	TGG	TTT	TCT	GGT	2352
Glu	Val	Met	Glu	Lys	Leu	Thr	Arg	Lys	Asn	Met	Asp	Trp	Phe	Ser	Gly	
	770					775					780					
GAA	AAC	AAC	AAG	GCT	TCT	GAA	ATT	TCT	GCT	CTA	GTC	TTG	AAT	GAT	TTG	2400
Glu	Asn	Asn	Lys	Ala	Ser	Glu	Ile	Ser	Ala	Leu	Val	Leu	Asn	Asp	Leu	
785					790					795					800	
CGA	GAT	TTG	AGG	TCT	TTG	GTC	TCT	GAA	TTT	TTG	AGT	GCA	ATG	ACT	AGT	2448
Arg	Asp	Leu	Arg	Ser	Leu	Val	Ser	Glu	Phe	Leu	Ser	Ala	Met	Thr	Ser	
				805					810					815		

FIG. 6D

21/23

TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	TCG	GGC	2496
Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	Ser	Gly	
			820					825					830			
TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	ATT	ATA	2544
Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	Ile	Ile	
		835					840					845				
TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	GAT	TAT	2592
Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	Asp	Tyr	
		850				855					860					
AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	GTC	ACT	2640
Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	Val	Thr	
					870					875					880	
AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	CAA	TTG	2688
Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	Gln	Leu	
				885				890						895		
ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	AAA	CGC	2736
Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	Lys	Arg	
			900					905					910			
GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	GTC	ATG	2784
Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	Val	Met	
			915				920					925				
TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	ATG	ATT	2832
Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr	Tyr	Gly	Leu	Lys	Leu	Val	Met	Ile	
		930				935					940					
CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	TTA	TTA	2880
His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	Leu	Leu	
					950					955					960	
TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	GAT	ATA	2928
Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	Asp	Ile	
				965					970					975		
AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	GAT	GAA	2976
Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	Asp	Glu	
			980					985					990			
AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	GTG	GGA	3024
Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	Val	Gly	
			995				1000					1005				
ATA	GCT	GGT	CAC	ACC	TAC	CAT	GGC	AAA	GAA	AAA	AAA	CCA	ATC	GTT	TCA	3072
Ile	Ala	Gly	His	Thr	Tyr	His	Gly	Lys	Glu	Lys	Lys	Pro	Ile	Val	Ser	
		1010				1015					1020					

FIG. 6E

22/23

ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC TTT GTT Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val 1025 1030 1035 1040	3120
TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA GGT ATC Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile 1045 1050 1055	3168
ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA TTA ACA Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Thr 1060 1065 1070	3216
AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT ACG GAC Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp 1075 1080 1085	3264
GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA GTG GTT Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val 1090 1095 1100	3312
GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC CTA ACT Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr 1105 1110 1115 1120	3360
TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT GCA TTA Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu 1125 1130 1135	3408
AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG GGC TTG Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu 1140 1145 1150	3456
GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG CAA AGG Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg 1155 1160 1165	3504
CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG ATT TTA Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu 1170 1175 1180	3552
GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC ATC AAT Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile Ile Asn 1185 1190 1195 1200	3600
GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT ATA ACG Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr 1205 1210 1215	3648
CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT CTT AAA His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys 1220 1225 1230	3696

FIG. 6F

23/23

GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	AAT	AAT	3744
Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	Asn	Asn	
		1235					1240					1245				
CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA			3786
Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*			
		1250				1255					1260					

FIG. 6G

INTERNATIONAL SEARCH REPORT

Application No
PCT/US 94/04379

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/62 C07K13/00 G01N33/50 C12N1/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CELLULAR BIOCHEMISTRY Supplement 16F; April 3-16, 1992 page 15 see abstract no. U 116 ---	1,2,6-8, 13-15
A	MOLECULAR BIOLOGY OF THE CELL vol. 3 , September 1992 page 192A Y. MCCLENDON ET AL. 'Analysis of cystic fibrosis-related mutations in the yeast Ste6 protein' see abstract no. 1116 --- -/--	1,15

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

9 September 1994

Date of mailing of the international search report

26 -09- 1994

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 94/04379

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CELL vol. 73, no. 2 , 23 April 1993 , CAMBRIDGE, MA US pages 335 - 346 JOHN L. TEEM ET AL. 'Identification of revertants for the cystic fibrosis deltaF508 mutation using STE6-CFTR chimeras in yeast' see summary see page 335, right column, paragraph 4 - page 336, right column, paragraph 2 see page 336, right column, paragraph 4 - page 338, left column, paragraph 1 see page 342, left column, paragraph 2 - right column, paragraph 1; figure 1 ---	1-15
P,X	BIOCHEMISTRY AND CELL BIOLOGY vol. 71, no. 11-2 , December 1993 page AXXV JOHN L. TEEM ET AL. 'The construction of STE6/CFTR chimeric transporters to define functional similarities between transmembrane domain segments' see abstract -----	1,7, 13-15

